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The Indian Botanical Society

(Formerly "The Journal of Indian Botany")

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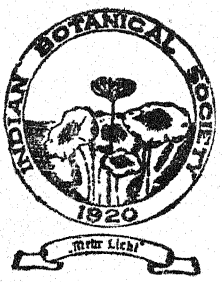

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THE EMBRYO-SAC OF *HECKERIA SUBPELTATA* KUNTH.

BY B. G. L. SWAMY

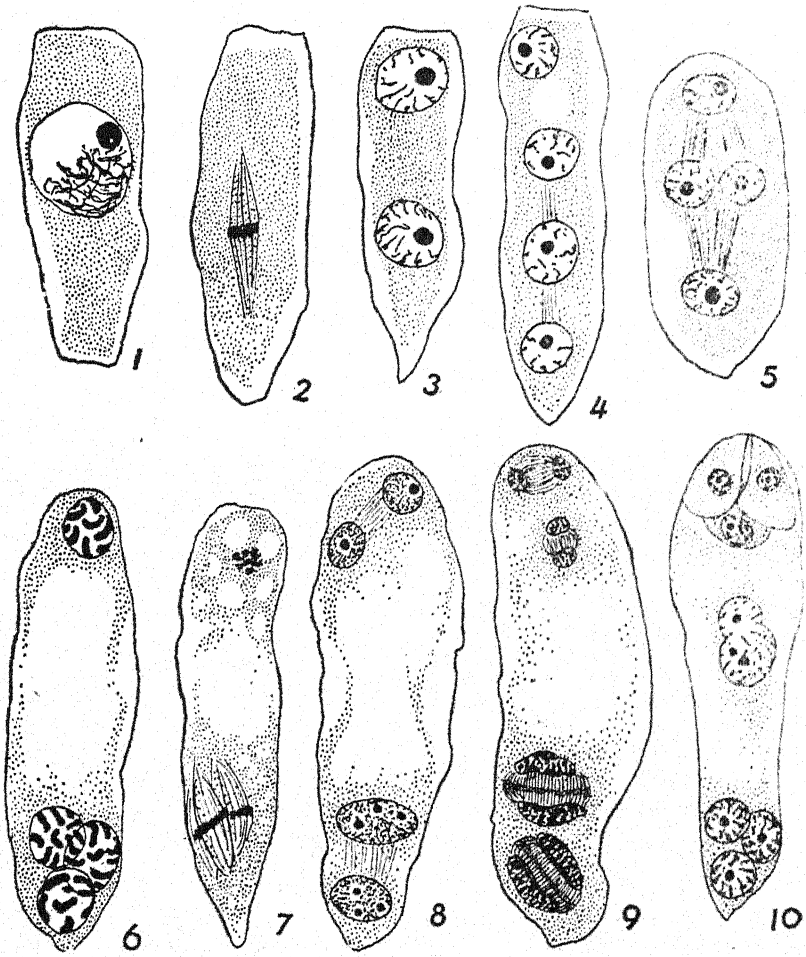
Received for publication on November 16, 1944

Heckeria umbellata and *H. peltata* were first investigated by Johnson (1902), who showed that the development of the embryo-sac in these species followed the *Adoxa*-type. Schnarf (1931, 1936) and Maheshwari (1937) made a careful study of Johnson's figures and opined that the *Fritillaria*-form would be the actual course of development of the female gametophyte. This surmise is borne out by a reinvestigation of *Heckeria umbellata* by Maheshwari and Gangulee (1942). *H. subpeltata* Kunth. is an uninvestigated species which grows in a wild condition in the evergreen forests of the Western Ghats. The results of a study of the development of the female gametophyte of this plant are presented in this paper.

OBSERVATIONS

The ovary and ovule present the same topographical and structural features as in *Heckeria umbellata* (Maheshwari and Gangulee, 1942). The archesporial cell is hypodermal in origin and cuts off a parietal cell, which divides only once in the majority of instances. The megaspore mother-cell enlarges and elongates lengthwise (Fig. 1). Its nucleus undergoes the characteristic pre-meiotic changes and divides into two nuclei (Figs. 2 and 3), which in turn complete the meiotic divisions by forming four megaspore nuclei which are not separated by walls ; the arrangement of the megaspore nuclei may be linear (Fig. 4) or more or less quadripolar (Fig. 5). At about this stage, one can notice a small vacuole appearing between the micropylar and the remaining three megaspore nuclei (Fig. 4). Finally the vacuole enlarges to such an extent so as to push away the micropylar nucleus and the three nuclei to opposite poles (Fig. 6). In this condition the nuclei begin to divide and the spindles of the three chalazally situated nuclei come still closer so that their equatorial regions lie more or less in a single line and plane (Fig. 7) ; but at the onset of anaphase, the

individuality of the three spindles is lost and they merge into a single large division figure, which produces two large triploid nuclei (Fig. 8). This stage of the embryo-sac, which shows two haploid nuclei at the micropylar and two triploid nuclei at the chalazal end, is the "Secondary four-nucleate stage". One more division of all the four nuclei (Fig. 9) results in an "8-nucleates" embryo-sac (Fig. 10), which,



Figs. 1–10.—Fig. 1. Megaspore mother-cell. Fig. 2. Division of the megaspore mother-cell. Fig. 3. 2-nucleate embryo-sac. Fig. 4. 4-nucleate embryo-sac in which the nuclei are arranged in a linear row. Note the vacuole between the micropylar nucleus and the rest. Fig. 5. 4-nucleate embryo-sac in which the nuclei are disposed in a quadripolar manner. Fig. 6. 1 plus 3 arrangement of the four nuclei. Fig. 7. Division of the "primary four nuclei" into the "secondary four-nucleate" embryo-sac; note the extremely juxtaposed spindles at the chalaza. Fig. 8. "Secondary four-nucleate embryo-sac." Fig. 9. The last (fourth) division of the embryo-sac nuclei. Fig. 10. Mature embryo-sac. All Figures × 900.

however, is equivalent to a tetrasporic 16-nucleate condition (*cf.* Swamy, 1944). The egg apparatus is organised by the haploid nuclei and the secondary embryo-sac nucleus is formed by the fusion of the haploid upper polar nucleus and the triploid lower polar nucleus.

SUMMARY

The female gametophyte of *Heckeria subpeltata* Kunth. has been studied and its development is shown to correspond to the *Fritillaria*-form.

LITERATURE CITED

Johnson, D. S. (1902) .. "On the development of certain Piperaceæ," *Bot. Gaz.*, **34**, 321–40.

Maheshwari, P. and Gangulee, H. (1942) "The development of the embryo-sac in *Heckeria umbellata* Kunth.," *Jour. Ind. Bot. Soc.*, **21**, 245–48.

Maheshwari, P. (1937) .. "A Critical Review of the types of Embryo-sacs in Angiosperms," *New Phyt.*, **36**, 359–417.

Schnarf, K. (1931) .. *Vergleichende Embryologie der Angiospermen*, Berlin.

———— (1936) .. "Contemporary understanding of the embryo-sac development among Angiosperms," *Bot. Rev.*, **2**, 565–85.

Swamy, B. G. L. (1944) .. "A Reinvestigation of the embryo-sac of *Piper betel* L.," *Proc. Nat. Acad. Sci. India*, **14**, 109–13.

A NOTE ON THE LIFE-HISTORY AND THE SYSTEMATIC POSITION OF *RHINOSPORIDIUM SEEBERI* (WERNICKE)

BY H. CHAUDHURI, BALBIR SINGH AND KARTAR SINGH THIND

Botany Department, Panjab University, Lahore

Received for publication on September 20, 1944

VARIOUS cases of rhinosporidiosis on man, horse and cow have been reported from all over the world. In India it has been observed in Bengal, Madras, Poona and other parts (Allen, 1935; Allen and Dave, 1936; Anantnarayan Rao, 1938; Beattie, 1906; Cherian and Vasu Devan, 1929; Karunaratne, 1936; Krishna Murti, 1931; Kurup, 1931; Mandlik, 1937; Noronha, 1933; Norrie, 1929; Sahai, 1938).

In the present case the rhinosporidiosis has been studied on cow, bullock and pony. The material was obtained by one of the authors (Balbir Singh) from various places in C.P. The nasal polypi of these animals along with their fæces and nasal excretions were fully studied. The microtomic sections of the polypi were also prepared.

The systematic position of the causal organism, *Rhinosporidium seeberi* has been so far a disputed question. An attempt has been made in the present publication to throw some light on this.

Young Stage

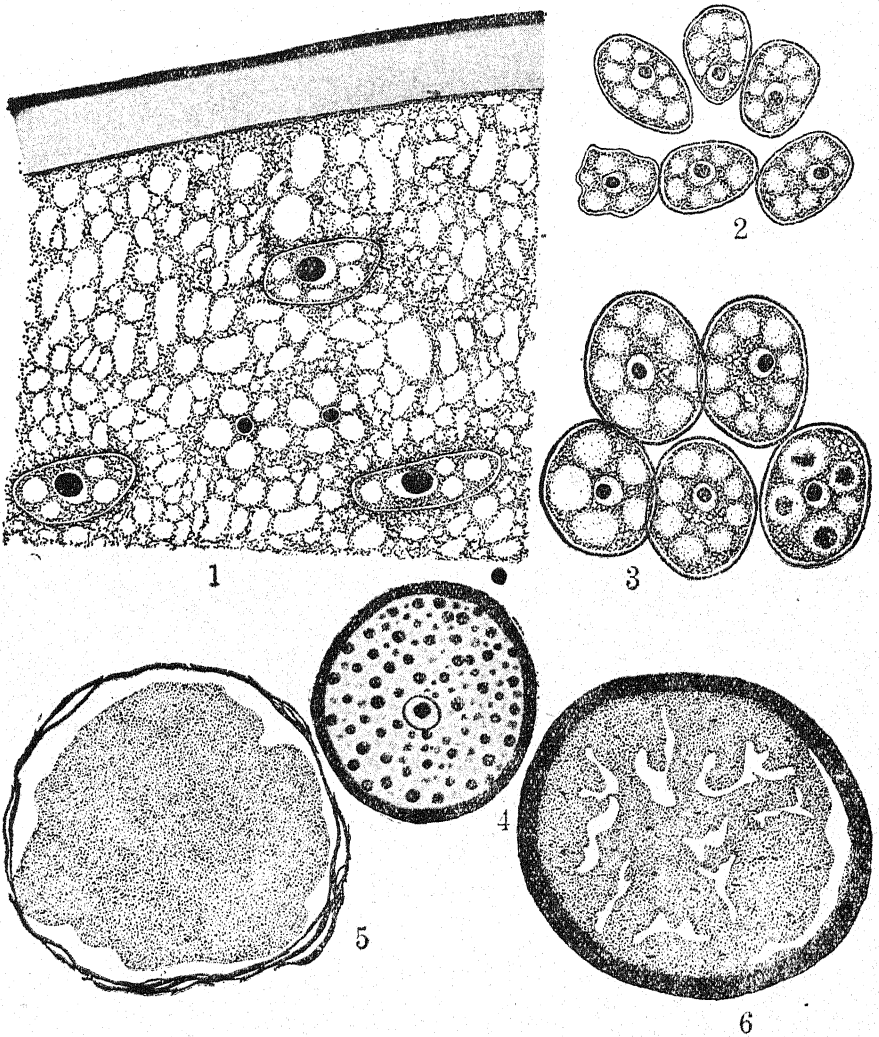
The parasite, as far as observed, starts its life-history with a small, spherical, oval or oblong body, sometimes with irregular boundary, inside the connective tissue cells of the polypus (Fig. 1). It measures 5–9 μ, the average being 6–8 μ in diameter. There is a nucleus with a distinct karyosome. The cytoplasm is granular and contains a few spherules (Figs. 2 and 3).

Trophic Stage

The parasite then enters a period of very active growth and considerably enlarges in size with corresponding accumulation of nutritive material in the form of spherical globules and increase in the size of the nucleus and the thickness of the wall (Figs. 4 and 12).

During the earlier part of the trophic stage the parasite remains more or less roundish and measures 13 μ to 65 μ in diameter. The wall is 1·3 μ to 5 μ in thickness and the size of the nucleus is 4 μ in a parasite which is 38 μ in diameter while it increases to 7 μ where the parasite attains the size of 60 μ in diameter. Later on the parasite becomes perfectly oval (Fig. 13) and measures from 91 μ to 130 $\mu \times 74 \mu$ to 78 μ. The thickness of the wall increases upto 9 μ as observed in a parasite

80 μ in diameter. In larger parasites the wall is comparatively thinner, being 7–8 μ thick.

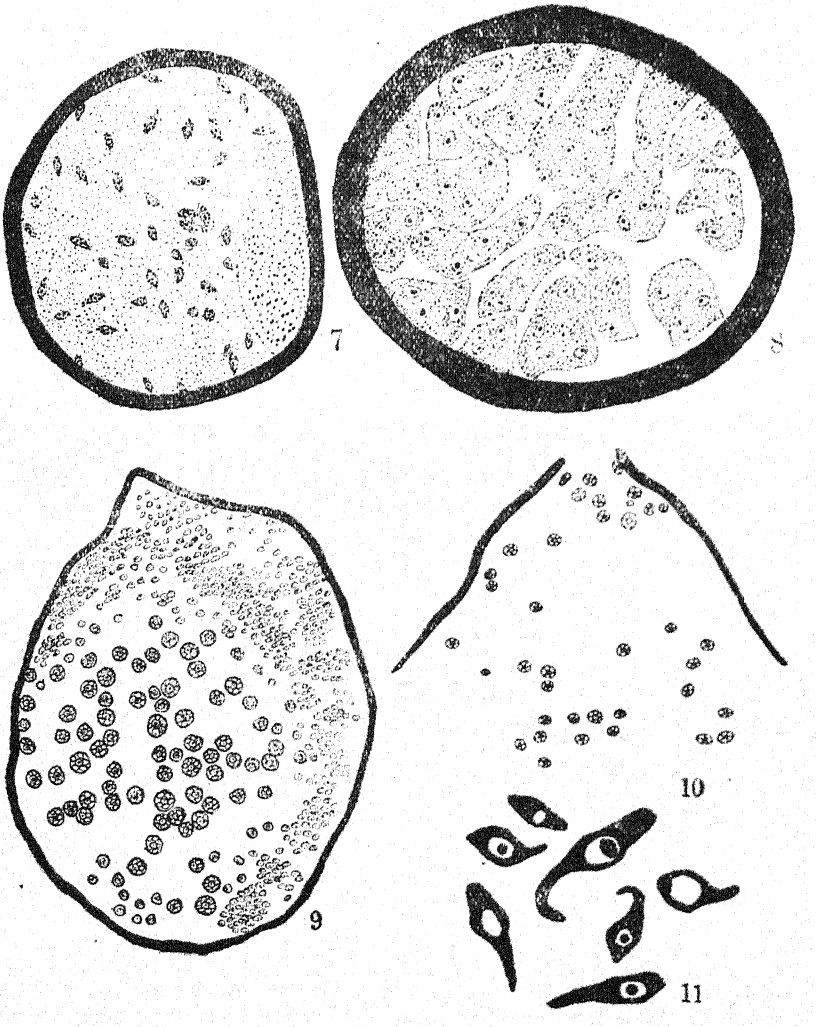


Figs. 1-6.—Fig. 1. Section of the polypus with spores in the connective tissue cells. Fig. 2. Young spores showing envelope, the nucleus with karyosome, the cytoplasm and the vacuoles. Fig. 3. Spores at a later stage of development than in Fig. 2. Fig. 4. Trophic stage. Fig. 5. Showing reduction in the size of the spherules and globules. Fig. 6. The nuclei formed after mitotic division. Figs. 1–3, × 2700; Fig. 4, × 1500; Figs. 5 and 6, × 510.

An important change now takes place in the size of granules and spherules, which were much larger in the beginning but become reduced in size later on, just prior to the nuclear division (Fig. 5).

Nuclear division

After the maximum growth period the nucleus of the parasite shows very active mitotic division. Several thousand nuclei are thus formed before the commencement of the cytoplasmic division (Figs. 6–7). The size of the parasite goes on enlarging and during the period of cytoplasmic division it varies from 144 μ to 109 μ × 130 μ to 90 μ. The thickness of the wall is from 4–9 μ.



Figs. 7–11.—Fig. 7. More nuclei formed after mitotic division. Fig. 8. Cytoplasmic division in progress. Fig. 9. Mature sporangium with beak at the top. Fig. 10. Pore differentiated at the top of the sporangium. Fig. 11. Germination of spores giving rise to amœboid structures. Figs. 7 and 8, × 510; Figs. 9 and 10, × 700; Fig. 11, × 1620.

Formation of Spores

Cytoplasmic division now sets in which is fully illustrated in Fig. 8. It goes on till there are formed uninucleate protoplasmic masses (Fig. 9). These round off and a wall is laid down around each. The parasite now represents a young sporangium with numerous uninucleate spores (Fig. 9). These young sporangia vary in size from 187 μ to 110 μ × 156 μ to 110 μ while the spores measure from 3–4 μ in diameter. It is interesting to note that the central spores are differentiated earlier than the peripheral ones (Fig. 9).

The sporangia and the spores further increase in size. The fully ripe sporangia measure from 500 μ to 400 μ × 400 μ, while the spores reach 6–9 μ in diameter. These measurements are much higher than those given by Ashworth (1924).

Dispersal of Spores

At any point the wall of the sporangium may protrude out into a beak (Fig. 9). The beak later on breaks and a pore is formed through which the spores are discharged (Fig. 10).

Germination of Spores

Numerous spores from which blunt processes were seen in all stages of development were observed by the authors (Fig. 11). The amœboid structures thus formed seem to be the germinating spores of *Rhinosporidium seeberi* and were found in the nasal excretions. These no doubt bring about new infection.

It has, however, not been possible to carry out the artificial germination of these spores.

Systematic Position of Rhinosporidium seeberi

This organism was first seen by Seeber in 1896 in nasal polypi of man in Buenos Aires, which he described as a sporozoal parasite (Seeber, 1900) and Wernicke named this parasite as *Coccidium seeberi* in 1900. Belou (1903) in his treatise on animal parasitology described it as *Coccidium seeberi* Wernicke, 1900. Minchin and Fantham described *Rhinosporidium kinealyi* as a new genus and a new species from nasal polypi in man from India. Beattie (1906) also described *Rhinosporidium kinealyi* from Cochin material, obtained by Dr. Nair of Madras. Seeber's parasite is a *Rhinosporidium* and is the same as *R. kinealyi*. Fantham, Stephens and Theobold (1916) call it *R. kinealyi* (or *seeberi*). The question of priority of name has been discussed by Seeber (1912) and as pointed out by Hartmann (1921) the *Rhinosporidium seeberi* Wernicke has priority over *R. kinealyi*. From the nasal septum of a horse in South Africa, Zschokke (1913) described *R. equi*, a new species. That there is any specific difference between the human and equine form seems doubtful (Wenyon, 1926). All these authors regarded *Rhinosporidium* as a protozoa. Ridewood and Fantham (Fantham, 1907) in their classification put *Rhinosporidium* in subsection Polysporulea under Haplosporidia. Doflein (1906) also retained it in

Polysporulea but stated that it had many resemblances with *Chytridineæ*. Ashworth (1923) conclusively demonstrated that these were vegetable parasites. He gave a detailed account, calling it *Rhinosporidium seeberi* and related it with lower fungi for the following reasons :

(1) Presence of fatty reserves, (2) repeated nuclear division preparatory to spore formation, (3) division of cytoplasm at a later stage, (4) absence of residual cytoplasm, (5) presence of a mucoid substance between the spores, (6) wall being made up of cellulose and (7) formation of a definite pore in the sporangium.

As the thallus in *Rhinosporidium* is formed of a single cell and the mycelium is wholly lacking, Ashworth put the organism under Chytridineæ. The thallus of *Rhinosporidium seeberi* is holocarpic, *i.e.*, later on gives as a whole to the sporongium. So he put it in the family *Olpidiaceæ* of Chytridineæ.

The occurrence of germinating spores giving rise to amœboid structures as observed by the authors, indicates the affinity of *Rhinosporidium seeberi* to Chytridiales. The formation of zoospores has been suppressed here probably due to its peculiar mode of existence on man and other animals (Negroni, 1931).

Ainsworth and Bisby (1943) wonder if *Rhinosporidium* be put under *Endomycetales*. But from the evidence put forward it appears that it should be placed under *Chytridiales*.

It may be mentioned here that Anantnarayan Rao (1938), in his paper while giving a brief account of the organism, refers to both sporangia and asci. It seems that he has confused the two terms.

SUMMARY

Rhinosporidiosis occurring on cow, bullock and pony has been studied. The fæces and the nasal excretions were also examined. The amœboid structures formed from the germination of spores of *Rhinosporidium seeberi* were found in the nasal excretions. These structures further strengthen the affinity of *R. seeberi* with *Chytridiales*.

The authors have great pleasure in acknowledging their thanks to Dr. G. Watts Padwick, Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi, for help with many references to literature and for kindly looking through the manuscript.

LITERATURE CITED

1. Ainsworth, G. C., and Bisby, G. R. (1943) — *Dictionary of Fungi.*
2. Allen, F. R. W. K. and Dave, M. L. (1936) — "The treatment of Rhinosporidiosis in man based on the study of sixty cases," *Ind. Med. Gaz.*, **71**, 7, 376-95, 5 figs.
3. Allen, F. R. W. K. (1935) — "Five cases of rhinosporidiosis—four in females," *ibid.*, **70**, 2, 76.
4. Anantnarayan Rao, M. (1938) — "Rhinosporidiosis in bovines in Madras Presidency, with a discussion on the probable mode of infection," *Indian Jour. Vet. Sci.*, **8**, 187–98, 4 pl.

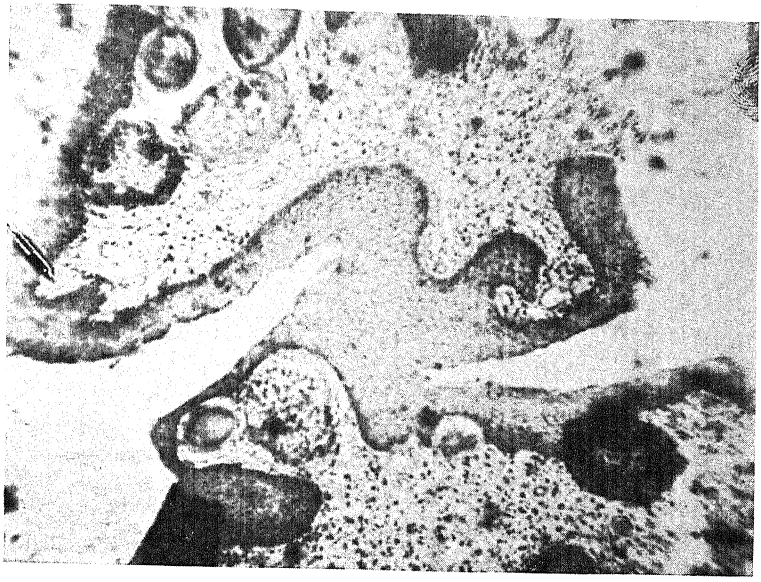


Fig. 12. Section of Polypus (trophic stage). ×95

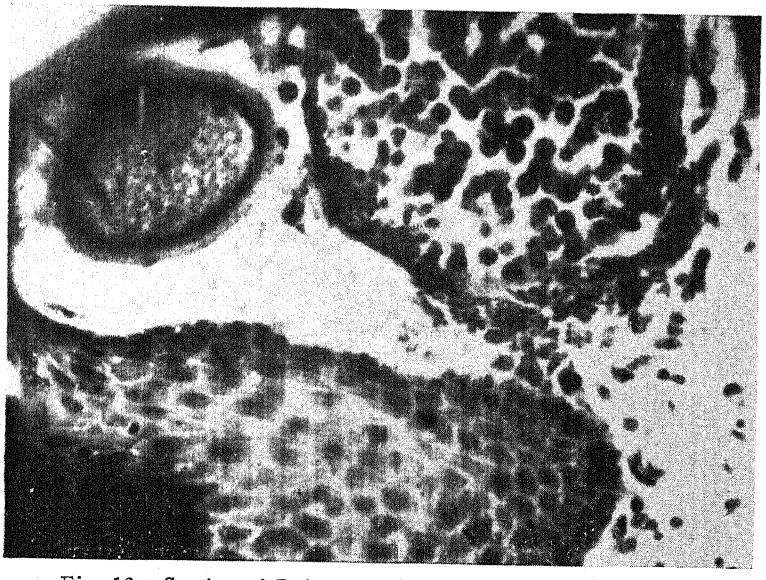


Fig. 13. Section of Polypus with mature sporangium. ×720

5. Ashworth, J. H. (1923) "On *Rhinosporidium seeberi* (Wernicke, 1903) with special reference to its sporulation and affinities," *Trans. Roy. Soc. Edinburgh*, **53**, 301–42.
6. Beattie, J. M. (1906) .. "*Rhinosporidium kinealyi*," *Journ. Path. Bacteriol.*, **11**, 270.
7. Belou, P. (1903) .. *Tratado de Parasitologia Animal*, Buenos Aires, 62.
8. Cherian, P. and Vasu Devan, A. (1929) "A case of rhinosporidiosis in the female," *Journ. Laryngl. and Otology*, **44**, 8, 518–19, 2 pl. (1 col.).
9. Doflein, F. (1916) .. *Lehrbuch der protozoenkunde*, 4th Edition.
10. Fantham, H. B. (1907) "The classification of Haplosporidia," *Rep. Brit. Asson.*, 553.
11. ———, Stephens, J. W. W. and Theobold, F. V. (1916) *The Animal Parasites of Man*, 197.
12. Hartmann, M. (1921) "*Rhinosporidium*," *Prowazek. u. Noller's Handb. d. path. Prot. Leipzig*, 1387.
13. Karunaratne, W. A. E. (1936) "The pathology of rhinosporidiosis," *J. Path. Bact.*, **42**, 1, 193–202, 7 pl. (1 col.).
14. Krishna Murti, V. (1931) "Rhinosporidiosis in Equines," *Indian Jour. Vet. Sci.*, **2**, 49–52, pl. 5.
15. Kurup, P. K. (1931) .. "*Rhinosporidium kinealyi* infection," *Ind. Med. Gaz.*, **66**, 5, 239–41, 4 figs.
16. Mandlik, G. S. (1937) "A record of rhinosporidial polypi with some observation on the mode of infection," *ibid.*, **72**, 3, 143–46.
17. Minchin, E. A., and Fantham, H. B. (1905) "*Rhinosporidium kinealyi* n.g., n.sp. A new sporozoon from the mucous membrane of the septum nasi of man," *Quart. Jour. Micro. Sci.*, **49**, 521–32.
18. Minchin, E. A. (1912) *An Introduction to the Study of Protozoa*, 424–25.
19. Negroni, P. (1931) .. "Estudio micologico sobre cincuenta casos de micosis observados en Buenos Aires," *Rev. Univ. Buenos Aires*, ser. 2, **29**, iv, viii, 3, 399, 1 col. pl., 75 figs.
20. Noronha, A. J. (1933) "A preliminary note on the prevalence of rhinosporidiosis among sand workers in Poona, with a brief description of some histological features of rhinosporidial polypus," *Jour. Trop. Med. Hyg.*, April 1933.
21. Norrie, F. H. B. (1929) "Rhinosporidium infection of the nose," *Jour. of Laryngology and Otology*, **44**, 8, 505–13.
22. Seeber, G. R. (1900) .. "Un Nuevo esporozoario parasito del hombre dos casos encontrados en polipos nasales," *Tesis Univ. Nac. de Buenos Aires*, 62, 3 pl.
23. ———— (1912) .. *Rhinosporidium kinealyi seeberi une question de priorite*, Buenos Aires, 19, 3 pls.
24. Sahai, L. (1938) .. "Rhinosporidiosis in equines," *Ind. J. Vet. Sci.*, **8**, 221–23, 2 pl.
25. Wenyon, C. N. (1926) *Protozoology*, **1**, 778.
26. Zschokke, E. (1913) .. "Ein *Rhinosporidium* beim Pferd," *Schweiz. Arch. Tierheilk*, **55**, 641.

STUDIES IN THE CÆSALPINIACEÆ

I. A Contribution to the Embryology of the Genus Cassia

By J. V. Pantulu

Maharaja's College, Vizianagram

Received for publication on September 22, 1944

Our present knowledge of the embryology and cytology of the three families Mimosaceæ, Cæsalpiniaceæ and Papilionaceæ belonging to the order Leguminosæ (if we follow the classification of Hutchinson, 1926), chiefly on account of their different distribution, is very unequal. The Papilionaceæ being cosmopolitan and abundant both in Europe and N. America have received much attention, while the more tropical Mimosaceæ and Cæsalpiniaceæ have been only meagrely investigated. This fact has prompted the author to take up the study of the Cæsalpiniaceæ. The present paper deals with the structure and development of the ovule and embryo-sac of *Cassia* species. The author has started with this genus not only because it is the largest in the family and is represented by many species in this country, but also because the few observations that have been made by the earlier workers are in several cases contradictory.

Previous Work

The earliest reference to the embryology of the Cæsalpiniaceæ is found in the work of Braun (1860), who observed polyembryony in a species of *Cassia*. Later Guignard (1881) in his extensive studies on the embryology of the Leguminosæ also made some observations on the genera *Cæsalpinia*, *Cassia*, *Cercis*, *Gleditschia* and *Ceratonia*. He observed in *Cercis siliquastrum* both the chalazal megaspores often becoming 2-nucleate and each having the capacity of developing into a mature embryo-sac. Some further observations on the embryo-sac of *Cassia* were made by Hubert (1896).

Saxton (1907) worked out the structure and development of the ovule and embryo-sac of *Cassia tomentosa*. He observed a deeply situated primary archesporial cell which functions as the megaspore-mother cell without cutting off any primary wall cell. The megaspore-mother cell undergoes the two meiotic divisions in the normal manner and forms a linear tetrad of megaspores. The second megaspore from the chalazal end develops into the embryo-sac according to the *Normal* type. The mature embryo-sac at the chalazal end forms a tubular-extension which becomes filled with a row of antipodal cells, as happens in some Compositæ.

Ghose and Alagh (1933) studied *Cassia purpurea*. They found hypodermal primary archesporial cell in the ovules and the formation

of a primary wall cell. The second megaspore from the chalazal end, as in *Cassia tomentosa*, was found to develop into the embryo-sac.

Datta (1935) investigated *Cassia tora*. He found in the ovules sub-hypodermal primary archesporial cells, absence of the primary wall cells, and organisation of a linear tetrad of megaspores, out of which the chalazal one developed into the 8-nucleate embryo-sac. The antipodals, even though they were found to persist till fertilisation, remain only as free nuclei and are not organised into cells.

The latest work on the embryo-sac of the Cæsalpiniaceæ is a paper by Paul (1937) dealing with *Tamarindus indica*. He reports the differentiation of the primary archesporium from the sub-hypodermal layer, formation of the primary wall cell and a normal tetrad of megaspores from the megaspore-mother cell. The chalazal megaspore is the functional one and develops into the embryo-sac according to the *Normal*-type.

Material and Methods

During the course of the present investigation material of the following species of Cassia has been investigated.

1. *C. occidentalis* Linn.
2. *C. abtusifolia* Linn.
3. *C. glauca* Lamk.
4. *C. glauca* Lamk. var. *suffruticosa* Koenig.
5. *C. marginata* Roxb.
6. *C. siamea* Lamk.

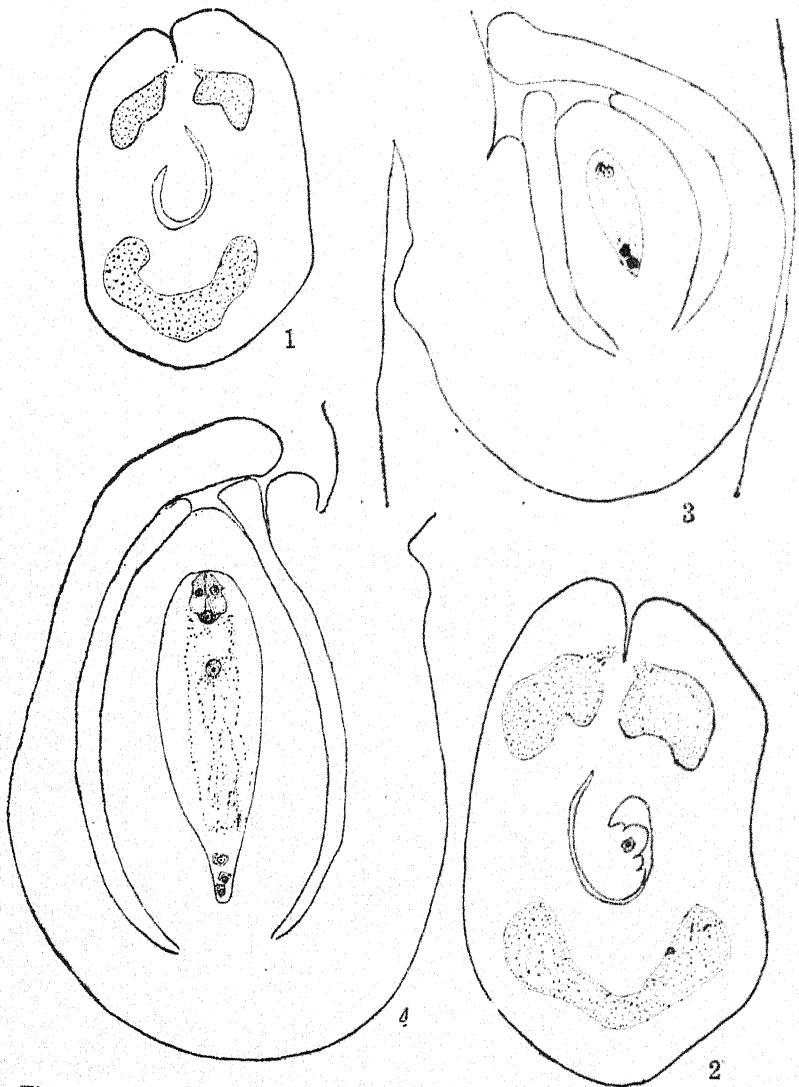
The first two species grow abundantly at Benares, particularly during the rainy season in waste places, and their material was collected from plants growing wild in the Benares Hindu University area. The material of *Cassia glauca* was obtained from a plant cultivated in the Benares Hindu University Botanical Garden, and that of *C. glauca* var. *suffruticosa* from a plant growing in the Sri Sita Ram Krishishala, Benares. The material of *C. marginata* was collected by Dr. A. C. Joshi from a tree growing in one of the gardens at Allahabad and that of *C. siamea* from trees planted on road-sides in the Benares Hindu University campus.

In all cases the material was fixed in Nawaschin's fixative between 12 noon and 3 p.m. during the months of September, October and November, 1940. An exhaust syringe was employed to cause the rapid immersion of the material in the fixative ; 12–18 hours after fixing, the material was rinsed in water four or five times and then transferred to 70% alcohol. The further dehydration and embedding in paraffin was carried out according to the customary methods. Sections were cut 8–16μ thick. Delafield's Hæmatoxylin and Newton's Iodine Gentian Violet were employed as stains.

Structure and Development of the Ovule

The ovules in all species of *Cassia* are borne in two rows along the ventral suture of the monocarpellary unilocular gynœcium, the ovules

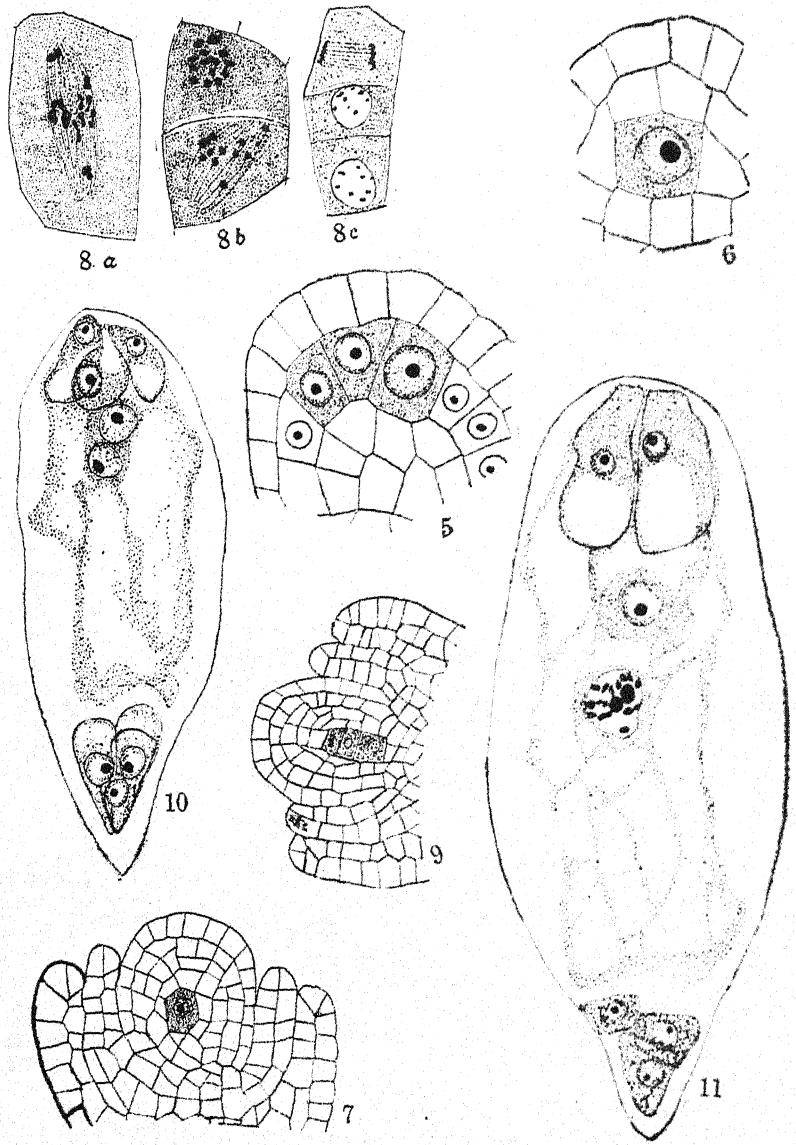
of the two rows alternating with one another. The number of ovules in a carpel varies slightly with each species, but it may be stated that on the average there are 30–50 ovules in a carpel.



Figs. 1–4. *Cassia occidentalis*.—Figs. 1 and 2. Transverse sections of young ovaries showing early stages in the development of the ovule. The vascular bundles of the carpel are stippled. Fig. 3. An ovule at the 4-nucleate embryo-sac stage. Fig. 4. An ovule at the mature embryo-sac stage. Figs. 1 and 2, × 800; Figs. 3 and 4, × 150.

The ovules first arise as small papillæ from the margins of the carpel, which has at this stage the form of a linear structure folded upwards along the midrib, a fact which agrees with the classical interpretation of carpel morphology. The two margins of the carpel are still free from each other and the carpel is open on the posterior side (Fig. 1). The development of the ovule primordia results chiefly from the activity and rapid division of groups of hypodermal cells, and after their differentiation these primordia are seen protruding into the ovary cavity (the space enclosed by the wall of the carpel). The ovule primordia at first are quite straight, but soon during further growth the cells develop more actively on one side than on the other. Consequently the young ovules bend towards the apex of the ovary, and gradually assume an anatropous form (Fig. 3). Reeves (1930) in *Medicago* observed that the curvature of the ovules is conditioned by mechanical pressure. He found as long as there is space for free development, the ovule remains orthotropous, but as soon as the ovule during its growth comes in contact with the dorsal wall of the carpel opposing it, its straight growth comes to end and it curves generally towards the base. Maheshwari (1931) in *Albizzia Lebbek* describes the young nucellus as growing at first straight and at right angles to the placenta (ventral suture), but when it approaches the dorsal wall of the carpel it begins to curve upwards. Singh and Shivapuri (1935) describe the same condition in *Neptunia oleracea*, a member of the Mimosaceæ. In a few cases, in which the carpel was found to remain open throughout its development, the ovules were found to remain permanently orthotropous. Great significance has been attached to this fact by Joshi (1935) in the evolution of the anatropous form of the ovule. In *Cassia* species studied during the course of the present investigation, however, no such relation has been found. The primordia of the ovules begin to bend towards the apex of the ovary even when these are quite away from the dorsal wall of the ovary.

The mature ovules in all *Cassia* species, even after the development of embryo, are anatropous with a slight tendency towards amphitropy (Figs. 3 and 4). They possess two integuments. The inner integument in the flowering plants generally differentiates from the ovule primordium almost simultaneously with the differentiation of the primary archesporium, but in all *Cassia* species investigated by the writer it did not appear till the primary archesporial cell had cut off the primary wall cell and had reached the megaspore-mother cell stage. The development of the integuments thus in the genus is considerably delayed. The primordium of the inner integument arises just below the level of the megaspore-mother cell (Fig. 2). Soon after its differentiation, the primordium of the outer integument appears just below that of the inner integument. In spite of the late start, the outer integument soon outgrows the inner by its faster development, so that by the time of tetrad formation the outer integument has attained a slightly greater length than the inner (Figs. 25 and 31). While the outer integument by this time has reached the level of the nucellus apex, the inner integument is seen to end somewhat below the level of the nucellus. The disparity between the growth of the two integuments is maintained



Figs. 5–11. *Cassia occidentalis.*—Various stages in the development of the embryo-sac.—Fig. 5. Apex of the nucellus showing a group of primary archesporial cells. Fig. 6. Formation of the primary wall cell and its division by an anticlinal wall. Fig. 7. An ovule showing the megaspore-mother cell. Fig. 8 *a–c.* Three stages in the development of a tetrad of megaspores; (*a*) I meiotic division in the megaspore-mother cell; (*b*) the II meiotic division; (*c*) a stage showing the formation of a T-shaped tetrad of megaspores. Fig. 9. The ovule from which the T-shaped tetrad shown in Fig. 8 *c* has been sketched. Fig. 10. A young 8-nucleate 7-celled embryo-sac. Fig. 11. A mature embryo-sac after the fusion of the polar nuclei. Figs. 7 and 9, × 800; the rest, × 1700.

even in the later stages, so that in the mature ovule (an ovule at the time of fertilisation) the micropyle is mostly formed by the outer integument. The inner integument contributes only to a very small length of the micropyle (Fig. 4). There are two further peculiarities of the micropyle. Firstly, the passage formed by the outer integument is not quite opposite to that formed by the inner. It is rather to one side, so that the micropyle is not straight but somewhat zig-zag. Secondly, at the micropyle the outer integument is never in direct contact with the inner integument. In this region there is always a small space between the two integuments. Both the integuments in all species are two layers of cells thick except near the micropyle, where both the integuments are 4–5 cells thick.

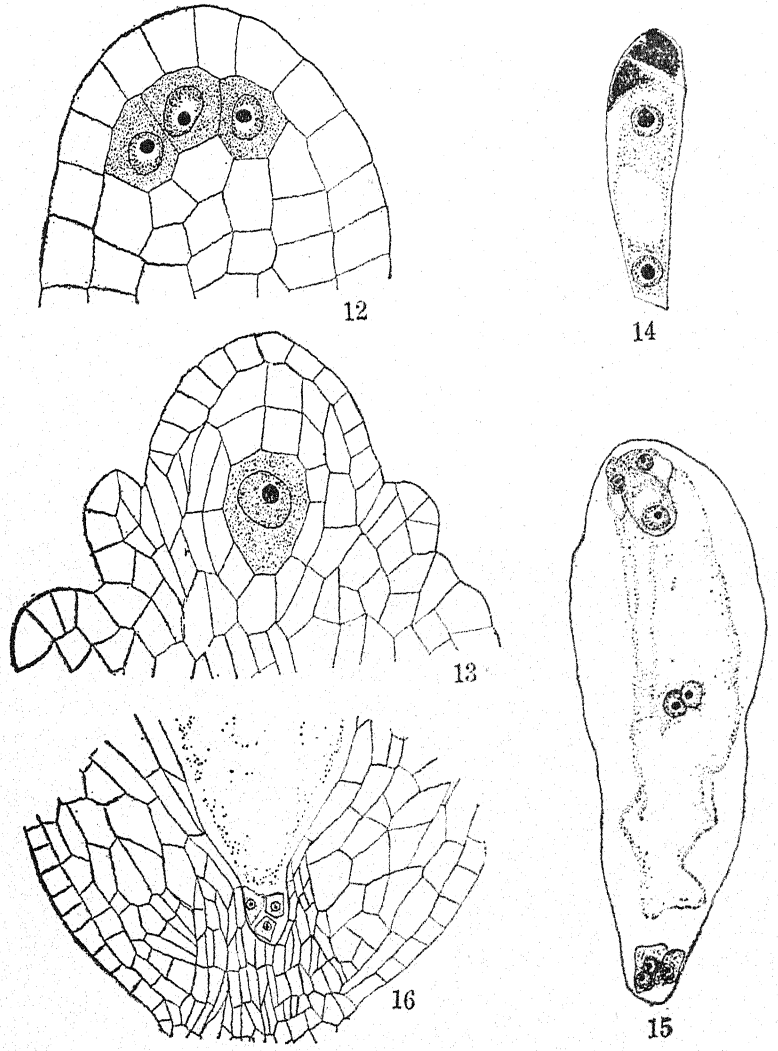
The nucellus in species of *Cassia* is massive from the very beginning. At the tetrad stage there are approximately 4–5 layers of nucellus cells above the tetrad, 3–5 layers on the sides, and 4–5 layers beneath the tetrad (Figs. 9 and 31). By the time the embryo-sac reaches the 4-nucleate stage the number of cells in the nucellus above the embryo-sac has increased to 8–10 layers due to divisions in the parietal cells. Before fertilisation many of these parietal cells are gradually crushed by the growing embryo-sac, but the number of cell layers above the micropylar end of the embryo-sac remains the same due to periclinal divisions in the epidermal cells of the nucellus. As the parietal cells are crushed at this end, the epidermal cells divide to restore the number of layers destroyed. This growth gives rise to considerable pressure inside the ovule, so that the epidermal cap shortly before fertilisation begins to project as a small beak into the micropyle of the ovule (Fig. 4). This pushes outwards the inner integument and leads to considerable decrease in the size of the air-space found between the two integuments close to the micropyle of the ovule. The formation just before fertilisation of an epidermal cap at the micropylar end of the nucellus with a small beak projecting into the micropyle seems to be a characteristic feature of all the Cassias examined by the author. I have seen it also in a number of other Cæsalpiniaceæ and perhaps this feature is characteristic of the whole family. There are approximately 6–7 layers of cells below the chalazal end of the embryo-sac, and 7–8 on the sides of the embryo-sac at the time of fertilisation. To a large extent these cell layers are soon crushed by the post-fertilisation growth of the embryo-sac.

Another characteristic feature of the ovule of Cassias is that the epidermis of the funicle and the adjacent part of the outer integument on the outer side (*i.e.*, the side on which the ovule does not bend) remains meristematic for a long time. At the tetrad stage these cells are quite distinct from the other cells of the ovule, possessing as they do dense cytoplasm and no conspicuous vacuoles. Further close to the hilum these cells grow out into a short hump-like outgrowth, which persists throughout the life of the ovule.

Development of the Embbyo-Sac

As the ovule begins to curve, but before the appearance of the integument primordia, the primary archesporium differentiates from

the other cells of the nucellus. In the flowering plants in general the curving of the ovule, the appearance of the integument initials and development of the primary archesporium are almost synchronous.



Figs. 12–16. [*Cassia obtusifolia*.—Fig. 12. [Nucellus showing a group of archesporial cells. Fig. 13. An ovule at the megaspore-mother cell stage. Fig. 14. A 2-nucleate embryo-sac, with three degenerating micropylar megaspores. Fig. 15. An 8-nucleate, 7-celled embryo-sac. Fig. 16. Antipodal region of an embryo-sac showing the tubular extension of its chalazal end. Fig. 15, × 1400; the rest, × 1700.

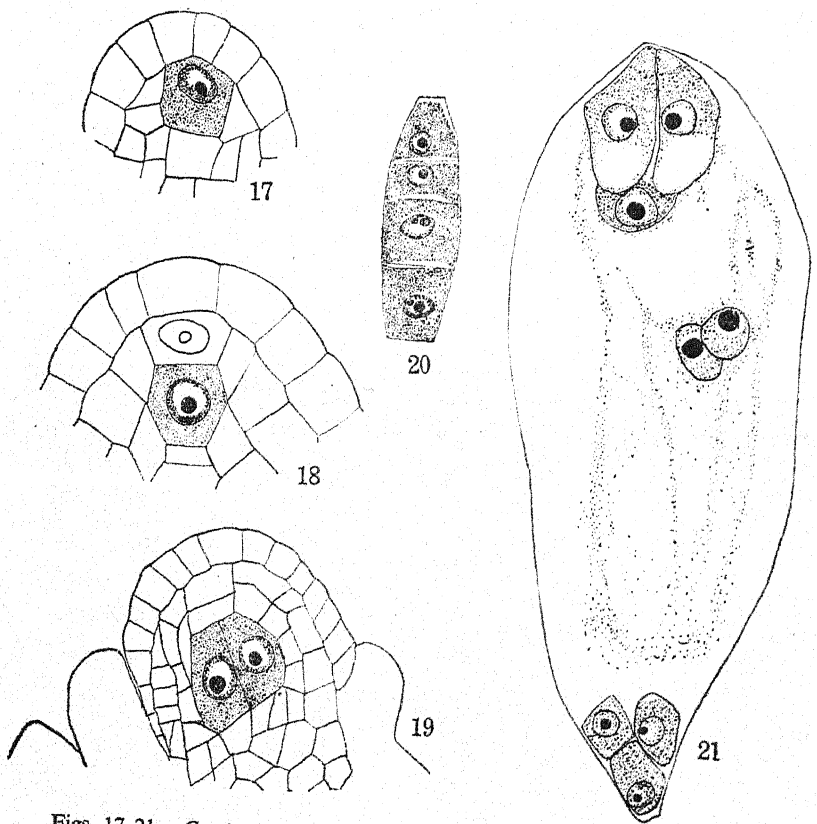
In all Cassias, however, as has been stated before, the integuments begin to develop rather late, only after the differentiation of the megaspore-mother cell in the ovules. The primary archesporium in all species

was found to be of hypodermal origin. In *Cassia glauca* and *C. glauca* var. *suffruticosa* a single primary archesporial cell is quite prominent from an early stage and can be easily distinguished from the surrounding cells (Figs. 17 and 22). In other species all the cells of the hypodermal layer are equally prominent and look just alike. They all show dense cytoplasm and possess almost equally large nuclei (Figs. 5, 12 and 26). One of these cells, however, generally the most centrally situated one, divides by a periclinal wall into an outer parietal cell and an inner megaspore-mother cell. This cell may be said to act as the primary archesporial cell (Figs. 6, 13, 18, 23, 29 and 30).

Describing the primary archesporium of the ovule, Coulter and Chamberlain (1903) state: "The archesporium is recognized by the increasing size and different reaction to stains of one or more hypodermal cells. Doubtless all of the hypodermal cells are potentially archesporial, and there is reason for believing that the deeper cells of the nucellus, most of which are probably derivatives from the original hypodermal layer, may be also. In the vast majority of the cases, however, only cells of the hypodermal layer show those changes that are characteristic of archesporial cells. It is not always easy to determine just how many hypodermal cells are to be included in the archesporium, for there is often complete gradation from cells with the size and staining reaction of undoubted archesporial cells to those showing neither increase in size nor the characteristic staining reaction. This is to be expected in case all the hypodermal cells are potentially archesporial, and there is no definite point in its history when such a cell ceases to be merely hypodermal and becomes clearly archesporial." While examining the ovules of the different species of *Cassia* for the primary archesporial stages, I have felt exactly like Coulter and Chamberlain. In the beginning in most species all the hypodermal cells at the apex of the nucellus are just similar. Then one of them cuts off a parietal cell and may be said to function as stated above as the primary archesporial cell.

Saxton (1907) noted in *Cassia tomentosa* that the primary archesporial cell is deep-seated, *i.e.*, sub-hypodermal and functions directly as the megaspore-mother cell without cutting off any parietal cell. Datta (1935) has described the same feature in *Cassia tora*. From the uniform hypodermal origin of the primary archesporium that I have noticed in the species examined by me, I am led to believe that the observations of both these authors are probably incorrect. This error has been made by them very likely from an examination of too old material, in which the parietal tissue had already begun to develop. I have not been able to see the paper by Saxton, but from examining the figures of Datta I find the Fig. 3 of his, which is almost at the same stage as Fig. 2 (and the latter is supposed to represent the primary archesporium). In the material examined by me the ovule has always developed up to the megaspore-mother cell stage by the time the integument primordia differentiate. Further, *Cassia obtusifolia* examined by me is very closely related to *C. tora*. In *Cassia obtusifolia*, I have clearly seen the hypodermal origin of the primary archesporium and the formation of a primary perietal

cell. It is not possible to believe that two such closely related species can show such a great difference in their embryological characters. What Datta regards as the primary archesporial cell is really the megaspore-mother cell after the cutting off of the primary wall cell. The observations of Ghose and Alagh (1930) on *Cassia purpurea* agree with mine. They also noted hypodermal archesporium and the formation of the primary wall cell. This character, therefore, may be taken as characteristic of the genus. Paul (1937) has reported sub-hypodermal origin of the primary archesporium in *Tamarindus indica*. I consider his observations also doubtful.



Figs. 17–21. *Cassia glauca*.—Fig. 17. The primary archesporial cell. Fig. 18. The differentiation of the primary wall cell. Fig. 19. An ovule showing two megaspore-mother cells. Fig. 20. A linear tetrad of megaspores. Fig. 21. A mature embryo-sac. Fig. 19, × 800; the rest, × 1700.

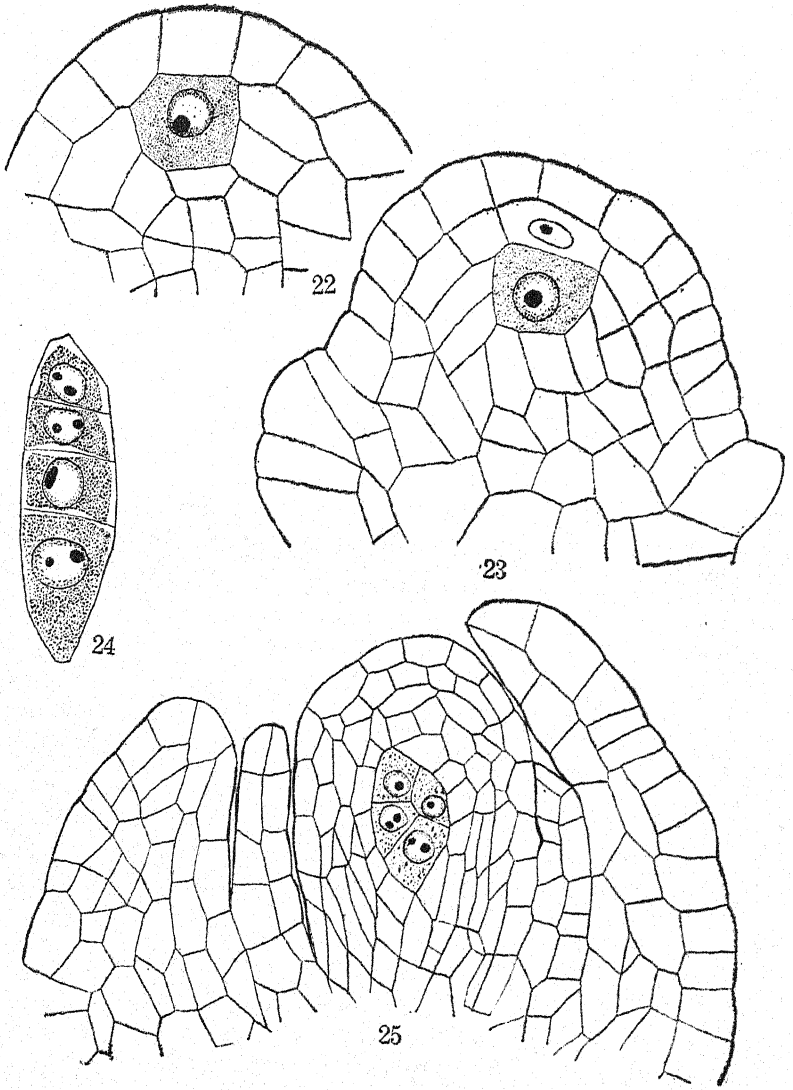
One functional archesporial cell and one megaspore-mother cell is the general character of the ovules of the different Cassias, but the occasional occurrence of two megaspore-mother cells has been observed in *Cassia glauca* (Fig. 19), *C. glauca* var. *suffruticosa* and *C. siamea*. Perhaps such exceptional cases are likely to occur in other species also

if a larger amount of material is examined. However, whenever two megaspore-mother cells were observed in an ovule, only one was seen to develop up to the tetrad stage. I did not come across any case of two tetrads or multiple embryo-sacs in an ovule. Occurrence of more than one megaspore-mother cells in an ovule has been previously noted by Datta (1935) in *Cassia tora*, and there are many similar instances reported among other Leguminosæ, *e.g.*, *Albizzia Lebbek* of the Mimosaceæ (Maheshwari, 1931), *Medicago sativa* (Reeves, 1930), *Melilotus alba* (Cooper, 1933), etc., belonging to the Papilionaceæ.

The primary parietal cell divides in all planes and by the time the two meiotic divisions in the megaspore-mother cell are completed, it gives rise to 4–5 layers of parietal cells (Figs. 9 and 31). Later the number of these layers increases to 8–10. Such extensive development of the parietal tissue seems to be characteristic of the Cæsalpiniaceæ and Mimosaceæ. In the Papilionaceæ, on the other hand, the parietal tissue is poorly developed. This agrees with the primitive character of the first two families and the more advanced position of the last family in the order.

The megaspore-mother cell after its differentiation undergoes a considerable period of rest and growth without any nuclear changes. It increases considerably both in length and breadth. The ovule also increases considerably during the megaspore-mother cell stage, so that the megaspore mother-cell becomes deep-seated. The meiotic divisions in all investigated species proceed in the normal manner. In *Cassia occidentalis* 14*n* chromosomes were counted during these divisions (Fig. 8 *a*). After the first meiotic division the mother cell is divided into two dyads by a transverse wall, which does not lie exactly in the middle (Figs. 8 *a*, *b* and *c*). The dyads are thus of unequal size, the chalazal one being larger. The second meiotic division in the two dyad cells generally does not proceed simultaneously. It starts earlier and proceeds more actively in the chalazal dyad than in the micropylar, so that in some cases even when the division has been completed in the chalazal dyad, the micropylar dyad is in the telophase stage (Fig. 8 *c*). Due to the difference in the size of the dyads, the megaspores formed from them also show slight size differences. The two chalazal megaspores are slightly larger than the two micropylar ones. The four megaspores are generally arranged in a linear order (Figs. 20, 24 and 31), but a T-shaped arrangement of the megaspores (Figs. 8 *c* and 9) was also seen in several instances in almost all species. In addition to this variation, in one ovule of *Cassia glauca* var. *suffruticosa* one megaspore-mother cell was observed to have given rise to an isobilateral tetrad of megaspores (Fig. 25). In this ovule there were two megaspores. One of these had formed this exceptional type of tetrad. The other was still in the megaspore-mother cell stage. It is not illustrated in the figure. Exceptional occurrence of isobilateral tetrads of megaspores in the flowering plants has been previously observed by Ducamp (1902) in *Fatsia japonica*, Greco (1930) in *Myrtus communis*, and Capoor (1937) in *Urginea indica*.

In all the species studied during the course of the present investigation the chalazal megaspore is found to develop into the embryo-sac (Figs. 14 and 31). The other megaspores degenerate, but the traces of the degenerating cells may be seen up to the 2-nucleate stage of the



Figs. 22–25. *Cassia glauca* var. *suffruticosa*.—Fig. 22. The primary archesporial cell. Fig. 23. Formation of the primary wall cell. Fig. 24. A linear tetrad of megaspores. Fig. 25. An ovule showing an isobilateral tetrad of megaspores. Fig. 25, × 900; the rest, × 1700.

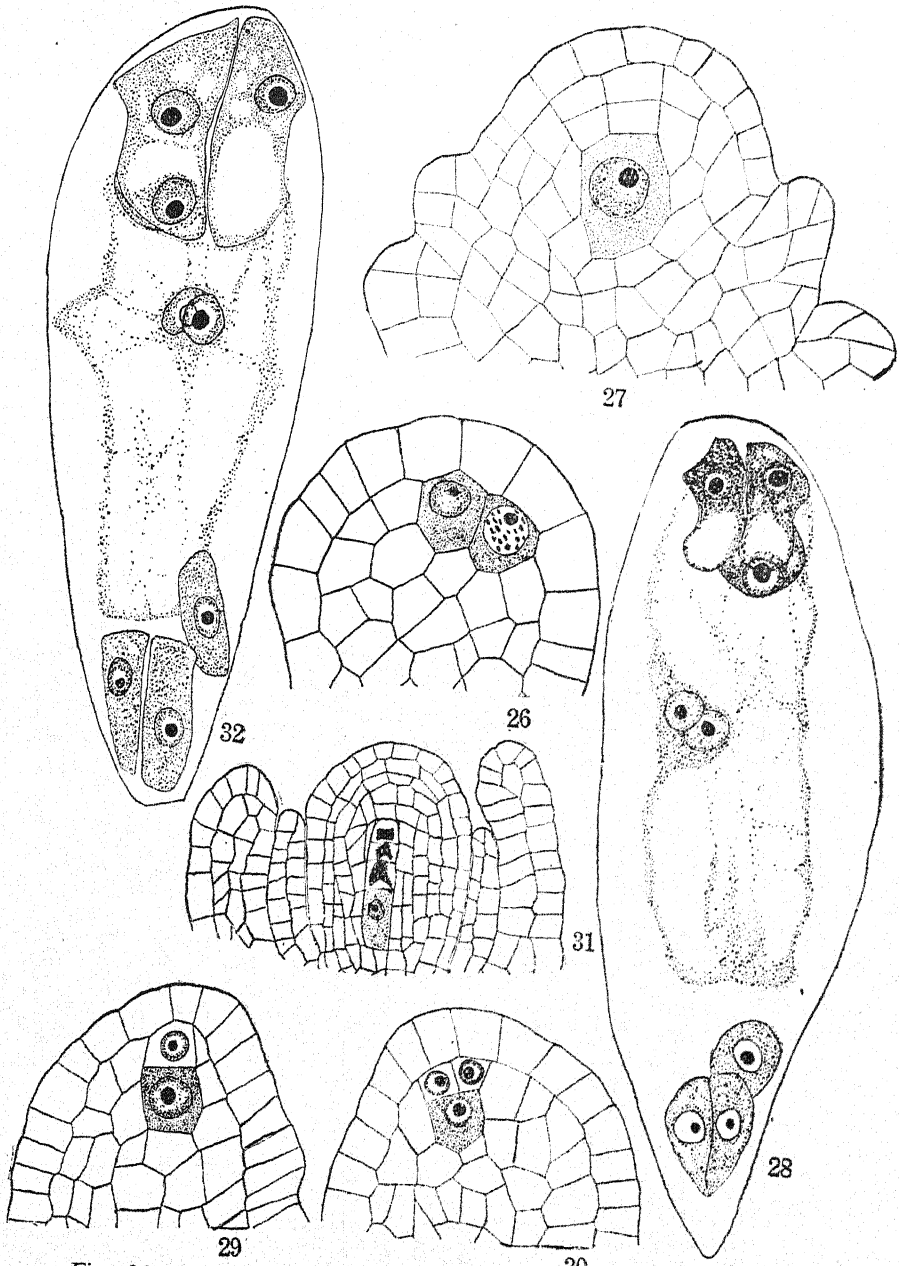
embryo-sac. Datta (1935) observed the same feature in *Cassia tora*, but Saxton (1907) and Ghose and Alagh (1933) found in *C. tomentosa* and *C. purpurea* respectively the second megaspore from the chalazal end developing into the embryo-sac. Such variation in the selection of the megaspores is common in the whole order Leguminosæ and was observed as early as 1881 by Guignard. From a study of about 40 species he concluded that in the Leguminosæ of the four megaspores of the tetrad either the innermost or the one next to it is the functional one.

The functional megaspore develops into the embryo-sac according to the *Normal*-type. It increases in size. Along with this vacuoles develop both above and below the central nucleus. The latter divides. The daughter nuclei move to the two poles of the embryo-sac and a central vacuole becomes prominent. The two nuclei at the poles undergo two more mitotic divisions, so that an 8-nucleate embryo-sac is formed with four nuclei at either end. Three nuclei at the micropylar end organise into the egg-apparatus, three at the chalazal end into antipodals and the two polar nuclei are left in the central cell (Figs. 10, 15, 21, 28 and 32).

Both the egg and the two synergidæ are nearly pyriform. The egg is slightly larger than the synergidæ. It shows a large vacuole towards the micropylar end, while the nucleus and the cytoplasm are pressed towards the chalazal end. The synergidæ show a large vacuole in the chalazal half, while the micropylar half is densely filled with cytoplasm. The nucleus is found embedded in the cytoplasm just above the vacuole. In all species the synergidæ show prominent hooks and a distinct "filiform apparatus" at the time of fertilisation (Figs. 11, 21, 28 and 32).

The antipodals form definite cells (Figs. 10, 11, 21, 28 and 32). Datta (1935) reports that in *Cassia tora* the antipodals are not organised into cells but remain as free nuclei. As I have observed antipodal cells in all the species investigated by me, his observations appear to me quite erroneous. Even in one of his own figures he has represented one of the antipodals as a cell with a cell-wall around it. In all the Leguminosæ investigated so far the organisation of antipodal cells has been noted. The antipodals in all Cassias are quite prominent and persist till the time of fertilisation. They often develop large vacuoles. In *Cassia glauca* var. *suffruticosa* they are sometimes even more prominent than the egg-apparatus. In *Cassia tomentosa*, Saxton (1907) mentions the presence of more than three antipodals, but I have not come across any such case in my material.

The two polar nuclei meet near the egg-apparatus or the middle of the embryo-sac. Here they remain together for a long time, but fuse only just before fertilisation. In *Cassia occidentalis*, the two polar nuclei just before fusion have been observed to enter the prophase stage and show the chromosomes quite distinctly (Fig. 11).



Figs. 26–32.—Figs. 26–28. *Cassia marginata.*—Fig. 26. A group of primary archesporial cells. Fig. 27. Apical region of an ovule with a megaspore-mother cell. Fig. 28. Mature embryo-sac. × 1700. Figs. 29–32. *Cassia siamea.*—Fig. 29. An ovule showing the differentiation of a primary wall cell and the megaspore-mother cell. Fig. 30. The same as Fig. 29 but showing the anticlinal division of the primary wall cell. Fig. 31. An ovule showing a linear tetrad of megaspores. Fig. 32. A mature embryo-sac. Fig. 31, × 900; the rest, × 1700.

SUMMARY

The development of the ovules and embryo-sac has been studied in *Cassia occidentalis* Linn., *C. obtusifolia* Linn., *C. glauca* Lamk., *C. glauca* Lamk. var. *suffruticosa* Koenig., *C. marginata* Roxb. and *C. siamea* Lamk. The ovules in all species are anatropous, with a slight tendency towards amphitropy, and bitegmic. The integument initials appear only after the primary archesporial cell has cut off the primary wall cell. The micropyle is somewhat zigzag and is formed largely by the outer integument. Further, in the region of the micropyle the outer integument for a short distance is separated from the inner by a small air-space. The nucellus is quite massive. The formation just before fertilisation of an epidermal cap at the micropylar end of the nucellus with a small beak projecting into the micropyle is characteristic. The epidermis of the funicle and the adjacent part of the outer integument on the outer side remains meristematic for a long time and close to the hilum grows out into a short hump-like structure, which persists throughout the life of the ovule.

The primary archesporium in all species is hypodermal and a primary wall cell is always formed. The earlier records about the occurrence of sub-hypodermal archesporium in some species of *Cassia* appear to be all doubtful. The megaspore-mother cell gives rise to a linear or T-shaped tetrad of megaspores, of which the chalazal develops into an 8-nucleate embryo-sac according to the normal type. In one instance in *C. glauca* var. *suffruticosa* an isobilateral tetrad of megaspores has been observed. The synergidæ are prominently hooked and show the filiform apparatus. The egg is pyriform and slightly larger than the synergidæ. The antipodals are definite cells and persist till the time of fertilisation. The two polar nuclei meet near the egg-apparatus. They fuse only just before fertilisation.

In conclusion I wish to express my sincere thanks to Dr. A. C. Joshi for his kind advice and help throughout the progress of the work.

LITERATURE CITED

Braun, A. (1860) .. "Über Polyembryony and Keimung von *Cælebogyne*," *Abh. Ak. Berlin, Phys. Kl.*, 1859, 107–263 (cited by Schnarf, 1931).

Capoor, S. P. (1937) .. "Contribution to the morphology of some Indian Liliaceæ. II. The gametophytes of *Urginea indica* Kunth.," *Beih. Bot. Zbl.*, **56**, 156–70.

Cooper, D. C. (1933) .. "Macrosporogenesis and embryology of *Melilotus*," *Bot. Gaz.*, **95**, 143–55.

Datta, R. M. (1934) .. "On the development of the embryo-sac and the pollen grain in *Cassia tora* Linn.," *Jour. Ind. Bot. Soc.*, **13**, 277–99.

Ducamp, L. (1902) .. "Recherches sur l'embryogénie des Araliacees," *Ann. Sci. Nat. Bot., Sér.* **8**, 15, 311–402.

Ghose, S. L. and Alagh, R. (1933) "Micro- and mega-sporogenesis in *Cassia purpurea* Roxb.," *Proc. Ind. Sci. Cong.*, 20*th Annual Meeting*, Patna.

Greco, R. (1930) .. "Embriologia del *Myrtus communis*," *Nuovo G. Bot. Ital.*, N.S., **38**, 609–30.

Guignard, L. (1881) .. "Recherches d'embryogénie végétale compareé. I., Legumineuses," *Ann. Sci. Nat. Bot., Sér.* **6**, 12, 5–166.

d'Hubert, E. (1896) .. "Recherches sur le sac embryonnaire des plantes grasses," *ibid.*, Sér. **8**, 2, 37–128.

Hutchinson, J. (1926) .. *Families of Flowering Plants. I. Dicotyledons*, London.

Joshi, A. C. (1935) .. "Criticism of Dr. Thomas's recent hypothesis on the nature of the angiospermous carpel," *Jour. Bot.*, **73**, 286–91.

Maheshwari, P. (1931) .. "Contribution to the morphology of *Albizzia Lebbek*," *Jour. Ind. Bot. Soc.*, **10**, 241–64.

Paul, A. K. (1937) .. "Development of the ovule and embryo-sac of *Tamarindus indica* Linn.," *ibid.*, **16**, 151–57.

Reeves, R. G. (1930) .. "Development of the ovule and embryo-sac of alfalfa," *Amer. Jour. Bot.*, **17**, 239–46.

Saxton, W. T. (1907) .. "On the development of the ovule and embryo-sac in *Cassia tomentosa*," *Trans. S. Afr. Phil. Soc.*, **18**, 1–5.

Schnarf, H. (1931) .. *Vergleichende Embryologie der Angiospermen*, Berlin.

Singh, B. and Shivapuri, T. N. (1935) "The gametophytes of *Neptunea oleracea* Lour" *Proc. Ind. Acad. Sci.*, B Ser. **1**, 421–34.

THE PLACE OF ANGIOSPERM EMBRYOLOGY IN RESEARCH AND TEACHING*

BY P. MAHESHWARI

Dacca University

IN the history of Angiosperm Embryology there have been three distinct periods : the *first* in which the chief aim was to unravel the fundamental facts regarding the development of the pollen and embryo-sac, and the processes of fertilisation and seed formation ; the *second* in which interest centred largely round a study of comparative embryology and an evaluation of the data thus obtained for the improvement of the existing systems of classification ; and the *third* and most recent in which Embryology has become an experimental science like Physiology and Cytology, where one tries to study such problems as the storage of pollen and its germination, the receptivity of the stigma, fertilisation and fruit-setting, etc., and the optimum conditions required for them.

DESCRIPTIVE EMBRYOLOGY

It is not necessary to spend much time on the first of these, *i.e.*, Descriptive Embryology, as most of the facts relating to the course of development of pollen, embryo-sac, endosperm and embryo had become clear towards the close of the last century through the efforts of Amici, Schleiden, Hofmeister, Strasburger, Treub, Guignard, Nawaschin and others, and are now a commonplace in all textbooks of botany. A very good summary of this work was given by Coulter and Chamberlain in the year 1903 and it was followed later by the publication of Schnarf's (1929) "Embryologie der Angiospermen", which is at present the most important and exhaustive treatise on this subject. Although little that is fundamentally new has probably been discovered since then, many errors and misinterpretations made by previous workers have been corrected and a mass of valuable information has been added regarding certain details concerned with the formation of the male gametes, the types of embryo-sac development, the cytology of fertilisation, the origin and function of endosperm haustoria and the development of the embryo. Work of this type is still in progress but the results will not be proportionate to the time spent unless a worker devotes his attention to just one aspect of the life-history in which he is most proficient and studies this in as many plants as possible. It is in this way that Finn in the Ukraine and Wulff in Germany and recently also some workers in the U.S.A. have been able to discover a number of important facts on the structure and development of the male gametes and Souèges in France on the development of the embryo in a large number of angiospermous families.

* Presidential Address delivered before the 24th Annual Meeting of the Indian Botanical Society held at Nagpur, on January 3rd, 1945.

Phylogenetic Embryology or Embryology in relation to Systematic Botany

In the second period, which may be said to have commenced with the beginning of this century, embryology began to be used as an aid in the improvement of our systems of classification, the most important contributions in this line having come from Sweden (Stockholm, Uppsala and Lund), Germany (Bonn, Berlin and Vienna), and the U.S.A. (Chicago, Baltimore and California). A great impetus was given to such studies by the publication of Schnarf's excellent handbook entitled "Vergleichende Embryologie der Angiospermen" in which the author has summarised the existing state of our knowledge of the embryology of each family and added a number of valuable suggestions and comments at the end of each order. Most of the embryological work done in India has followed the publication of this book, to whose author we owe a debt of gratitude which cannot be expressed adequately in words. Although I alone among the Indian embryologists have had the privilege of working with Prof. Schnarf, yet all have gained considerable inspiration and insight into this difficult field through the medium of his publications.

As the value of embryology in questions relating to systematic botany does not appear to be sufficiently appreciated in this country by those who are engaged in other lines of study, it is necessary to consider this aspect in some detail.

It is a matter of common knowledge that on the basis of external morphology of the vegetative organs a genetical relationship may sometimes be inferred to exist between plants which belong to widely separated groups (*Equisetum* and *Casuarina*; *Ephedra* and certain Asclepiads; some cacti and Euphorbias). Taxonomists therefore take recourse to the flower as it is a more conservative organ than the stem and leaf. But, if we assume phyletic trends in the external morphology of the flower, why not in the internal structures, for these must be still more conservative (being less amenable to environmental influences) and therefore of special value in judging the proper position of certain doubtful groups? I am told that the zoologist would ordinarily refuse to assign an unknown animal to its systematic position until he has had an opportunity of examining its internal organs. No one can doubt that the same should be done with plants also, and if anything has prevented *us* from applying the anatomical and embryological method on a large scale, it is only the greater labour involved in it. The work *has* however to be undertaken now on a larger scale than ever as the systematist has taken us almost as far as he could towards our goal of a natural system of classification and can hardly make much headway without our help and co-operation.

Before proceeding further I must now enumerate such characters in the embryology of an angiosperm which are usually considered to be of major value in delimiting the larger plant groups :—

1. *Anther tapetum.*—Whether it is of the glandular or the amœboid type.

2. *Quadripartition of the microspore mother-cell.*—Whether it takes place by furrowing or by the formation of cell-plates.

3. *Development and organisation of the male gametophyte.*—Number and position of the germ pores and furrows ; adornments of the exine ; place of formation of the generative cell ; number and shape of the nuclei in the pollen grain at the time of its discharge from the anther.

4. *Development and structure of the ovule.*—Number of integuments and the alterations in structure which they undergo during the formation of the seed ; presence or absence of vascular bundles in the integuments ; shape of the micropyle, whether it is formed by the inner integument or the outer or both ; the presence or absence of an obturator.

5. *Form and extent of the nucellus.*—Whether it is broad and massive or thin and ephemeral; presence or absence of a hypostase; the place of origin of the integument or integuments, whether close to the apex of the nucellus (as in the Rubiaceæ) or near its base (as in the Orchidaceæ) ; persistence or disappearance of the nucellus in the seed.

6. *Origin and extent of the sporogenous tissue in the ovule.*—Nature of the archesporium, whether it is one-celled or many-celled ; presence or absence of wall layers ; the presence or absence of periclinal divisions in the cells of the nucellar epidermis.

7. *Megasporogenesis and development of the embryo-sac.*—*i.e.*, to which of the following main types or its modifications does it correspond : Normal, *Oenothera*, *Allium*, *Peperomia*, *Fritillaria*, *Adoxa*, *Plumbago*, *Plumbagella*, etc. ?

8. *Form and organisation of the mature embryo-sac.*—Shape of the embryo-sac and the number and distribution of its nuclei ; an early disappearance or otherwise of the synergids and antipodal cells ; increase in number of antipodal cells, if any ; formation of haustoria, if any, from some part of the embryo-sac.

9. *Fertilisation.*—The path of entry of the pollen tube ; the interval between pollination and fertilisation ; any tendency towards a branching of the pollen tubes during their course to the ovule.

10. *Endosperm.*—Whether it is of the nuclear, cellular or Helobiales type, and direction of laying down of the first wall in such cases where it is cellular ; presence or absence of endosperm haustoria and the manner in which they are formed if present ; nature of food reserves in endosperm cells.

11. *Embryo.*—Relation of the proembryonal cells to the body regions of the embryo ; form and organisation of the mature embryo ; presence or absence of suspensor haustoria.

12. *Certain abnormalities of development.*—Apomixis, polyembryony, parthenogenesis, etc.

While these are the most important characters usually taken into account in systematic studies, there are many others which it is

difficult to put down in writing. Indeed, as a very competent embryologist (Mauritzon, 1939) recently remarked, the resemblances and differences in the embryological characters of the members of a family are sometimes of such a fine type, that they can neither be brought out in words nor even in a drawing but can only be appreciated under the microscope. He nevertheless considers them to be of distinct value in delimiting the smaller groups and in determining their interrelationships with one another.

Let us now take some specific instances where embryology has rendered an important service in the determination of the proper position of some difficult families or in giving a new orientation to our ideas of their affinities.

The relationships of the family *Empetraceæ* exercised the minds of systematists for a long time and it has been placed by some authorities in the Monochlamydeæ, and by others in the Sapindales or the Celastrales. Samuelsson's work (1913) has definitely shown however that its proper place is with the Bicornes, a group which is characterised by the following well-marked embryological features :—

1. Absence of a fibrous layer in the anthers.

2. Presence of a glandular tapetum which does not become amœboid.

3. Pollen grains remaining together in tetrads.

4. Ovule with a single integument and a thin ephemeral nucellus which completely disappears in later stages so that the embryo-sac lies in direct contact with the integumentary tapetum.

5. Absence of parietal cells in the ovule, the hypodermal archesporial cell functioning directly as the megaspore mother-cell.

6. Embryo-sac of the monosporic eight-nucleate type with small ephemeral antipodals.

7. A hollow and fluted style which connects the lumen of the ovary with the outside and along which the pollen tubes make their way into the ovary.

8. Endosperm cellular, the first two divisions being transverse and giving rise to a row of four cells placed above one another.

9. The formation of endosperm haustoria at both ends of the embryo-sac, micropylar as well as chalazal.

10. A single-layered seed-coat formed from the outermost layer of the integument, the remaining layers becoming absorbed during the growth of the embryo-sac and embryo.

All these are perfectly standard stages in Erican embryology, a combination of which is not known to occur in any other order except the Bicornes. The Empetraceæ show a close correspondence in all respects, while the Sapindales and Celastrales differ from them (Bicornes) in so many ways that Samuelsson may be said to have established his point of view fully and completely. Hutchinson's assignment of the Empetraceæ to the Celastrales is therefore considered

by Schnarf (1933, p. 283) to be due to nothing but a "ganz besonderer Verständnislosigkeit ".

On the other hand, the Lennoaceæ, which have sometimes been placed in the Bicornes (Hutchinson, 1926) certainly do not belong here. In the very first place, the equality in number of their stamens and corolla-lobes (contrasted with the obdiplostemony of the Ericales), the alternation of the parts, the adnation of the filaments to the corolla and the dehiscence of the anthers by longitudinal slits, form a weighty objection against this view. Add to these the fact that the Lennoaceæ have a short and solid style, a normally developed endothecium, pollen grains separate from each other, and a seed-coat which is more than one-layered. Svensson (1923) and Copeland (1935) therefore correctly consider the assignment of the Lennoaceæ to the Bicornes as quite untenable on embryological as well as other grounds and suggest that they might more reasonably be placed among the Tubiflorales as a separate suborder occupying a primitive position.

Let us now pass on to another group, the Cactaceæ. F. Vaupel (1925), in the latest edition of *Engler-Prantl's Pflanzenfamilien*, writes that there is hardly a family in the plant kingdom, the allocation of which has allowed so much scope to individual tastes as this. Wettstein placed it in the Centrospermales and Engler-Prantl in a separate order Opuntiales near the Passifloraceæ. Hutchinson has erected the order Cactales and placed it closest to the Cucurbitales.

The views of the great Viennese systematist have received very definite confirmation in this respect from the works of two embryologists, Mauritzon (1934) and Neumann (1935). Although additional work on this family would be welcome, the following features seem to be well established :—

1. A secretory tapetum of parietal origin.
2. Division of pollen mother-cells of simultaneous type.
3. Pollen grains tri-nucleate at the time of shedding.
4. Ovules campylotropous with strongly curved and massive nucelli.
5. Two integuments ; and the swollen lips of the inner, which alone forms the micropyle, protruding out to approach the funiculus.
6. A hypodermal archesporial cell which cuts off a wall cell.
7. A nucellar cap formed by periclinal divisions of the cells of the nucellar epidermis.
8. Embryo-sac of the Normal type.

Several other characters, for which a reference may be made to Frl. Neumann's original paper, point to the conclusion that the Cactaceæ belong to the Centrospermales and form a sort of bridge between the Aizoaceæ and Portulacaceæ. An interesting point, which has probably been overlooked by several workers but is nevertheless of considerable importance is the presence, in the chalazal part of the ovule, of a "Hohlraum" or "Luftspalt" between the two integuments and sometimes also between the inner integument and the nucellus.

This is quite distinctive of a number of other families belonging to the Centrospermales and its occurrence in the Cactaceæ is therefore of great significance.

On the other hand, a comparison of the embryology of the Cactaceæ with that of the Passifloraceæ offers so little by way of resemblance that any close relationship between them appears to be most unlikely.

Take again the Onagraceæ, in which the genus *Trapa* has long been considered to occupy a somewhat anomalous position. All the plants of this family so far investigated show a monosporic four-nucleate embryo-sac, *Trapa* alone being an exception with an eight-nucleate embryo-sac and a well-developed suspensor haustorium. From the embryologist's standpoint this strongly supports the case for a removal of this genus to a separate family—a course which has now been adopted by some systematists by erecting the family Hydrocaryaceæ for its reception. Regarding the relationships of the Onagraceæ with other families of the order Myrtales, it seems very likely that it has been derived from the Lythraceæ (Tischler, 1917) in which the ephemeral antipodals show the way to a complete omission of the chalazal part of the embryo-sac, leading to the four-nucleate condition of the Onagraceæ. I understand that this view is not challenged by systematists.

In Prof. Schnarf's laboratory at Vienna, some very important work has been done on the embryology of the Liliaceæ and Amaryllidaceæ, which has a great bearing on the interrelationships of the various sub-families and tribes included under these large and difficult families.

Taking the sub-family Lilioideæ, Engler (1888) divided it into the tribes Tulipeæ and Scilleæ. Schnarf (1929) stressed the sharp embryological differences between them, and in the second edition of the "Natürlichen Pflanzenfamilien" Engler and Prantl (1931) removed the Scilleæ from the association giving it the status of an independent sub-family, the Scilloideæ, so that the name Lilioideæ is now synonymous with the former Tulipeæ. Frl. Rosalie Wunderlich, a pupil of Prof. Schnarf, has again (1937) stressed the great contrast between the two (see table below) and even hinted at the desirability of separating them into two distinct families. She rightly points out that in more than one respect the Lilioideæ appear to be a derived group while the Scilloideæ are more primitive ; the latter should therefore be placed *before* the former and not after as it has been done by Engler and Prantl.

Dr. Wunderlich further adds that the Scilloideæ themselves fall into two tribes : one with the Helobiales type of endosperm (*Ornithogalum*, *Muscari* and *Puschkinea*) which she calls the *Ornithogalum* group and the other with the *Nuclear* type including the genera *Hyacinthus*, *Scilla*, *Camassia* and *Galtonia*.

	Scilloideæ	*Lilioideæ*
1.	Parietal cell always present in ovule	Parietal cell absent
2.	Embryo-sac of *Normal* or sometimes *Allium* type	Embryo-sac of *Fritillaria* type

	Scilloideæ	*Lilioideæ*
3.	Endosperm of *Nuclear* or *Helobiales* type	Endosperm of *Nuclear* type
4.	Embryo large, occupying almost the entire length of the seed	Embryo small (*Tulipa*, *Lilium*, *Fritillaria*, *Erythronium*, etc.) and occupies only a small space in the seed
5.	Generative cell small and slender, not easily stainable with acetocarmine	Generative cell large and broadly spindle-shaped, staining easily with acetocarmine
6.	Male nuclei ± spherical	Male nuclei ± elongated
7.	Chromosome no. variable	Chromosome no. usually 12
8.	Raphides present	Raphides absent
9.	Septal nectaries present	Septal nectaries absent

The systematic position of the Moringaceæ has long been a matter of some doubt. The astonishing observations of Rutgers (1923) on the embryo-sac and embryo of *Moringa oleifera* only increased this element of uncertainty. He reported in this plant the absence of a parietal cell in the ovule and the presence of a five-nucleate embryo-sac and a free nuclear embryo. My pupil, Prof. V. Puri of Meerut (1940), has shown that a parietal cell is *present*, the embryo-sac is of the normal *eight-nucleate* type and what Rutgers considered to be a free nuclear embryo is merely a group of some *endosperm nuclei* at the micropylar end of the sac, the fertilised egg having escaped his notice altogether! The resemblances which the Moringaceæ show to the Capparidaceæ in embryology and carpel morphology make it seem fairly certain that their correct position is in the order Rhœadales and the place assigned to this family by Hutchinson—between the Capparidaceæ and Tovariaceæ—is therefore justified.

Although provisionally placed with the Rosales, there has always been some doubt regarding the interrelationships of the Podostomaceæ with the other families of this order. The extensive work on the embryology of the Crassulaceæ and Saxifragaceæ done a few years ago by Mauritzon (1933) has however brought out certain features which make it almost certain that the Podostomaceæ are much reduced apetalous derivatives of the Crassulaceæ. The peculiar structure of the ovules of the former appears to be brought about merely as the result of a continuation of the reduction already seen in the Crassulaceæ and *Crassula aquatica*, in particular, whose mode of life is somewhat similar to that of the Podostomaceæ and which has the most reduced endosperm in the Crassulaceæ, may well form a transitional stage leading to the complete suppression of this tissue in the Podostomaceæ. A striking agreement between the two families is the presence of a highly developed suspensor haustorium, a feature which in Mauritzon's (1939, p. 38) opinion offers "such an eloquent proof" of their relationship "as to convert many doubters".

It is possible to add numerous other instances where embryology has rendered signal service to systematic botany, but considerations of time and space forbid me from citing them here. I believe that a stage has now arrived when we should try to have an embryological formula for each family as a supplement to the well-known floral formula so commonly used by systematists. To make my meaning clear I give below the embryological formula of the family Alismaceæ with which I have been particularly familiar as the result of the work done on it by my pupil Dr. B. M. Johri of Agra and later by Balwant Singh and myself (Maheshwari and Singh, 1943) at Dacca :—

ANTH.-TAP. (amœboid) ; DIV. OF P.M.C. (succ.) ; P. (3-nucl.) ; OVULE (2-integ. ; anat.) ; PAR. CELL (absent) ; E.S. (*Allium* T.) ; END. (*He.* or *Nu.* T.) ; EMB. (*Sag.* T.).

Put in plain English this means that the anther tapetum is of the amœboid type ; the divisions of the pollen mother-cells are successive ; the pollen grains are 3-nucleate at the time of shedding ; the ovule is anatropous and has two integuments ; no wall cell is cut off by the primary archesporial cell which functions directly as the megaspore mother-cell ; the embryo-sac development is of the *Allium* type ; the endosperm is of the *Nuclear* or *Helobiales* type ; and the embryo is of the *Sagittaria* type.

It may be possible by means of further abbreviations to include this information in a still shorter space and to make other improvements so as to devise a symbolisation which will be internationally acceptable. I suggest that this point may be discussed, by those who are interested, in the pages of one of our monthly journals like *Current Science*.

The embryologist would however be glad to admit that he lays no claim to erect a phylogenetic scheme of his own. Indeed there are some very definite limitations to the embryological method, for, owing to parallel, convergent and regressive evolution, similar embryological characters may often be found in widely separated groups and if a system of classification were to be set up on such considerations alone, some rather fantastic results are bound to ensue. But, with the main lines of phylogenetic classification already chalked out by the systematist, it is possible for the embryologist, the cytologist and the anatomist to use this as a background and to help him in making it more perfect. A natural system has to be discovered (for it is already there) and not invented. In order to do this we have to do real detective work and take the aid of every branch of botany. Once a group has been assigned to its true place, every character that is studied will only serve to strengthen its position. On the other hand, if there are any discrepancies, they will be brought out in a more glaring fashion by the study of its internal structures (as these are less influenced by the environment) than the external.

I understand that in some of the big herbaria of the U.S.A. steps are being taken to have along with the dried specimen a preparation or two showing the structural features of its wood. This is a laudable effort, but it ought to be extended still further so as to include

in each case about half a dozen preparations of the pollen, ovule and seed as well.

Applied and Experimental Embryology

Now we come to Applied Embryology which has evolved, for the most part, only during the last two decades or so. It is hardly possible to do any justice here to this subject, for although still in its infancy, it already has such a voluminous literature as to defy any attempt to review it in a few pages. I shall therefore satisfy myself merely by indicating some of the main lines on which work is being carried on in this field.

Before any improvement of our crop plants can be undertaken through breeding methods, it is necessary to have *in each case* a thorough understanding of the behaviour of the flower throughout its development and the setting of the fruit. Of the greatest importance in this connection are :—a study of the viability of the pollen and the optimum conditions for its storage and germination ; the receptivity of the stigma ; the rate of pollen tube growth under different conditions of temperature and humidity ; the interval between pollination and fertilisation and how it can be influenced by external conditions ; and the quantity of pollen necessary for a proper fruit set. Considerable work of this nature is being done in America and Russia, regarding the immense value of which in our breeding programmes there can be no question.

It is said that the Arabs put aside the pollen of the date palm from year to year so as to ensure a supply of dates even in the possible event of the male flowers failing to develop or the female flowers developing precociously. If we succeed in devising suitable conditions of temperature and humidity for storing the pollen of other cultivated plants, which normally is not so long-lived, we may be able to cross two varieties which flower on widely different dates or which are separated by considerable distances from each other. In the latter case it may be possible to transport the pollen by air from one place to another. We may hopefully envisage the possibility of opening one or more "pollen banks" in each country, where pollen of almost every important plant of economic value will be stored under optimum conditions and supplied to recognised workers, gratis or on a moderate charge.

Sterility and unfruitfulness are often caused by a very slow growth of the pollen tube. The flowers do not remain attached to the plant for an indefinite period and unless fertilisation takes place within a reasonable time, varying with the species under consideration, abscission takes place at the base of the style and fruit setting is consequently prevented. Premature as well as delayed pollination have the same result and we therefore need full information regarding all of our fruit trees and crop plants on the rate of pollen tube growth and the time when the stigma is most receptive. The optimum conditions for the germination of pollen also need to be investigated more fully. Tischler's (1910) discovery that much of the pollen of certain species of *Cassia* occurring at Buitenzorg fails to develop without an

outside supply of diastase illustrates the need of such work from various points of view.

Another aspect of applied embryology is a study of the possibility of obtaining a fruit set without the generally associated seed formation. There are a number of our edible fruits where the pericarp is the chief edible portion and the presence of seeds is neither necessary nor desirable. It was found possible in several cases to do away with the fertilisation of the egg cell and give the ovary the necessary stimulus for further development by the application of pollen extracts. This led to a chemical analysis of the latter which in turn opened the way for the induction of artificial parthenocarpy through the use of growth hormones (for literature see Maheshwari, 1940; Gustafson, 1942).

A very important paper was published in 1928 by C. A. Jørgensen, which showed the way to the induction of parthenogenesis in flowering plants. He pollinated the stigmas of *Solanum nigrum* with pollen from some other species of this genus. Most of the fruits were seedless but a few were found to have formed 2 to 8 seeds which gave rise to haploid plants of *Solanum nigrum*. An embryological study showed that in certain cases the foreign pollen had germinated successfully and the pollen tubes had also reached the embryo-sacs, but the male nucleus which enters the egg cell eventually disintegrates and disappears. The egg cell thus develops by itself into the embryo, stimulated no doubt by the entry of the male nucleus. The plants produced from the resulting seeds are therefore haploids resembling the maternal parent. In other cases, apparently, the male gamete alone may give rise to the embryo as inferred from the characters of the offspring which resembles the paternal parent but as far as I am aware the cytological and embryological processes leading to this condition are still unknown. Jørgensen's work opened the way towards the artificial production of haploids in a number of species and races. Although weak and valueless in themselves, they are of great utility in giving us an insight into the genetic constitution of the parent variety and for the production of homozygous diploids by a subsequent doubling of the chromosomes.

The effects of X-rays, colchicine treatment and exposure to extreme temperatures on the normal course of development are other aspects of recent cytoembryological research, which it is impossible to deal with here. It need only be said that while a fair amount of work has been done on the manner in which they influence ordinary mitotic and meiotic divisions, we are still very much in the dark about their effect on megasporogenesis, fertilisation and the development of other ovular structures like the endosperm and the embryo.*

The Place of Embryology in Botanical Teaching

At the end of this very short and imperfect sketch of the aims and scope of embryological research we may now consider another aspect of the subject, *e.g.*, its place in botanical teaching. That a study of embryology demands a more thorough training in microtechnique

* The important work, which is being done by Blakeslee and others on the artificial culture of excised embryos, will be reviewed elsewhere.

than is usually needed for other branches of plant science is a fact well known to everybody and it is perhaps for this reason that while much time is spent in our class-rooms on vegetative anatomy, in several universities little or nothing is shown to the students concerning angiosperm life-histories. A great opportunity is lost thereby of training them in the art of observation, reconstruction and critical interpretation. While granting that it is the advent of the microtome, as an instrument of precision in making serial sections, that has done so much for the recent progress in this science, it is not to be imagined that an elaborate scheme of fixing, dehydration, infiltration, imbedding, cutting, and staining is necessary in all cases. Much can be seen and shown by simpler methods. All stages of microsporogenesis and the maturation of pollen grains can be observed by making smears of suitable materials like *Tradescantia*, *Gloriosa*, etc., stained with either gentian violet or Feulgen (for technique see Darlington and La Cour, 1942). It is possible to mount the pollen grains of *Ottelia* and *Hydrilla* in acetocarmine and make a preparation, showing the vegetative and generative (or sperm) cells, even under the low power, in less than 5 minutes. Anthers of even herbarium specimens are often quite usable for such purposes (see Leitner, 1938). Some years ago Dr. Wulff and I (Maheshwari and Wulff, 1937) gave a schedule for making permanent mounts of pollen tubes to show the division of the generative cell and the organisation of the male cells. The common garden species of *Impatiens* is very good for this purpose as the pollen grains germinate readily and show the desired stages in only half an hour's time. Some of the Pontederiaceæ like *Monochoria* and perhaps several other plants may be equally suitable for the purpose. With the use of vital stains such slide cultures of pollen tubes may be used for studying the movement of the cytoplasm and the male gametes.

An observation of the stages in megasporogenesis and embryo-sac formation involves greater difficulties as the cells concerned are encased in several layers of other cells belonging to the nucellus and integuments, but Hillary (1940) has recently developed a technique by which he has been able to follow the development of the embryo-sac of *Lilium longiflorum* right from the megaspore mother cell up to the time of fertilisation and beyond, without cutting any sections. The ovules are here taken out from the ovary and the tissue around them removed as far as possible. Then they are fixed, washed, and stained with Feulgen's reagent in small vials or tubes. From the SO_2 water they are transferred to a drop of acetic acid placed on a slide and crushed under a coverslip. The author presents photomicrographs made from such preparations, which show the nuclei and chromosomes standing out quite distinctly in the colourless cytoplasm of the embryo-sac.

In his work on *Notonia grandiflora*, Ganesan (1939) used a somewhat similar method in order to select material of the right age for a study of the reduction divisions in the ovule. In this case an ovule is dissected out and mounted in a drop of acetocarmine mixed with an equal quantity of 1% safranin in 50% alcohol. By gentle and gradual increase of pressure on the coverglass, the nucellus is now freed from the

thick integument and in about half an hour's time the megaspore mother-cell nucleus is adequately stained for the purpose. By this method the author was able to exercise some judgment at the time of fixing the material and save much labour which would otherwise have been wasted if the cutting had been done at random. Poddubnaja-Arnoldi's (1938) "rapid method of embryological investigations" is essentially similar except that she recommends a mixture of acetocarmine and glycerine. She was thus able to follow the development in some plants upto the first stages in the formation of the embryo.

Suitable material for watching the process of fertilisation without recourse to section cutting has not yet been found* but one may try for this purpose such plants as *Torenia* and *Utricularia* in which the nucellus degenerates early and the upper part of the embryo-sac protrudes out of the micropyle so that it is naked and therefore more readily observable. The styles and stigmas of *Portulaca*, *Ottelia* or *Monochoria* should also be examined in order to follow the course of the pollen tube from the stigma to the ovule. A treatment with lactophenol and cotton blue often facilitates such observation.

That certain interesting features of endosperm morphology can be brought out more clearly from whole mounts of suitably dissected material than from sections, is shown by the work of Kausik (1939) on *Grevillea*. Dr. Kausik discovered in this plant a curious "worm-like" structure, which he calls the "vermiform appendage", formed by the chalazal part of the endosperm. This was missed by earlier observers since they used only sections which naturally fail to give any complete or intelligible picture of this tortuous organ.

There is perhaps no way of studying the development of the embryo except by cutting thin sections of the ovule but favourable material may yet be discovered in which the technique so successfully used by Buchholz (1938) in the study of conifer embryogeny will be found adaptable for at least some stages of this process. Also, in certain cases the seed coat may be so transparent (B. G. L. Swamy tells me that this is the case in many orchids) that it is possible to see the embryo in whole mounts of the seed without recourse to sectioning.

Let me explain why I am so keen that students should cut sections and make whole mounts or try other methods so as to see the entire process of development of the gametophytes and embryo in angiosperms as an essential part of any course in botany. This is because there are few other spheres of botanical study which offer a similar variety of technical problems or opportunities for the development of a critical attitude which is the most important quality that a young worker must learn to imbibe. I hope to be excused for citing here the case of a student who, having just taken the Master's degree, placed before me with great satisfaction a set of slides in which he claimed to have seen "all" stages of the development of the embryo-sac—1-nucleate, 2-nucleate, 3-nucleate, 4-nucleate, 5-nucleate, 6-nucleate,

* *Monotropa*, which is said to be very favourable for this purpose, is unfortunately not available in this country except in the hills and other inaccessible places, and the plant is not amenable to cultivation owing to its saprophytic habit.

7-nucleate and 8-nucleate. When the preparations were scrutinised it was found that all the sections were of mature embryo-sacs, but as the nuclei were spread apart into several sections, they were counted as they came, some here and others there, and imagined to be *stages in development* of an embryo-sac. Again, scores of students get away through a university course with the impression, gained from a study of book figures, that the integuments are lateral processes developing from the right and left sides of the nucellus, although a cross-section of the ovule or a whole mount of the same would have easily convinced them that it is not so.

I should add that it is not merely the student who makes mistakes but that even the experienced researcher is liable to be misled into such erroneous interpretations as may in some cases ruin his reputation as a scientific worker. Indeed, there are so many pitfalls in the correct interpretation of the material that the embryologist must always remain as watchful and alert as the worker on fossils. As an instance may first be cited the case of the *Lilium* embryo-sac whose development was repeatedly and very intensively studied by the most competent workers like Strasburger, Coulter, Mottier, Guignard and others. And yet, all of them were mistaken as the excellent work of Bambacioni (1928) showed a few years later. The embryo-sac of *Plumbagella*, long considered to be the most reduced among angiosperms, has turned out to be but a modification of the type seen in *Fritillaria* and *Lilium* (Fagerlind, 1938 ; Boyes, 1939). *Plumbago* has also been shown to have a development very different from that originally described for it by Dahlgren (see Haupt, 1934) and this has now been confirmed for another member of the same family, *Vogelia indica*, found in Rajputana (Mathur and Khan, 1941). In *Euphorbia heterophylla*, Sanchez (1938) recently reported a tetrasporic embryo-sac which has on reinvestigation turned out to be of the normal monosporic type (Maheshwari, 1942). Then again, the embryo-sac of *Rudbeckia*, which Palm (1934) believed to be of an entirely new type has been found to correspond with the now well-known *Fritillaria* type (Maheshwari and Srinivasan, 1944).

Another kind of error which is of frequent occurrence is the mistaking of the integument for the nucellus or *vice versa*. Even so recently as 1938 Houk fell into such an error in the case of the ovule of *Coffea* and in his confusion stated that the tissue may be regarded as an "integument-nucellus". Joshi (1938), Mendes (1941) and several other workers have shown that the nucellus and integument are both formed normally but the former soon disappears as is usual in most Sympetalæ. A similar mistake appears to have been made by Pannochia-Laj (1938) who writes that in *Lochnera rosea* the ovule is peculiar in that it is not possible here to delimit the nucellus from the integument. In another genus, *Fouquieria*, the structure which was supposed to be a " massive " nucellus is really the inner integument, the former being extremely reduced and ephemeral (see Khan, 1943). Evidently Dr. Woodcock (1943) has also been misled when he says that in *Ipomœa rubro-cærulea* the ovule "has no distinct integument" and the micropyle is formed by an "invagination at the end of the ovule next to the funiculus (see Maheshwari, 1944*b*).

The origin of the haustorial processes in the ovule has been another fruitful source of errors and misinterpretations. To mention only two such cases, Heinricher (1931–32), in his monograph on the genus *Lathræa*, stated that the micropylar haustoria are formed from the synergids and the chalazal from the antipodal cells. This was promptly contradicted and disproved by Glišic' (1932) who made a thorough study of *Lathræa squamaria* and found that both the haustoria are formed from the endosperm. A similar mistake made by G. O. Cooper (1942), working on *Lobelia cardinalis*, has already been commented on by me a few months ago (Maheshwari, 1944*a*).

Without citing further instances, I shall conclude this portion of my address merely by saying that even if the laboratory work in this subject makes greater demands upon the energy and resourcefulness of the teacher, this should not be grudged, as through this the young pupil gets such a stimulus for his mental development as is sure to be of use to him ever afterwards in his future career.

The Future

And now we proceed to the future.

It is said by some that the days of descriptive embryology are now over. This is far from true in my opinion. We need more of such investigations and will continue to do so for a long time. What needs to be emphasized, however, is that the descriptions must be full and accurate and the interpretations checked as critically as possible with preparations of the highest quality. As I have mentioned earlier, results of greater value may be expected if attention is focussed on a comparative study of only one aspect of the life-history at a time, *viz.*, male gametophyte, ovule, embryo-sac, pollen tube, etc. Each of these requires the study of a vast amount of literature and sometimes a technique different from that used for the rest.

With respect to phylogenetic embryology there is a great scope in our country, for we have representatives of a number of families in India, Burma and Ceylon, which have either received little or no attention or which deserve more intensive study than has so far been bestowed upon them. I mention below the names of a few but the list is by no means exhaustive and is capable of amplification :—

Aristolochiaceæ
Balanophoraceæ
Berberidaceæ
Burseraceæ
Callitrichaceæ
Ceratophyllaceæ
Cornaceæ
Crypteroniaceæ
Dilleniaceæ
Dipterocarpaceæ
Droseraceæ
Ebenaceæ
Flacourtiaceæ
Fumariaceæ
Hippocrateaceæ
Loranthaceæ
Magnoliaceæ
Myristicaceæ
Myrsinaceæ
Nepenthaceæ
Nymphæaceæ
Pittosporaceæ
Podostemaceæ
Salvadoraceæ
Simarubaceæ
Sterculiaceæ
Symplocaceæ
Thymelæaceæ
Zygophyllaceæ
Cyperaceæ
Dioscoreaceæ
Eriocaulaceæ
Flagellariaceæ
Hæmodoraceæ
Juncaceæ
Lemnaceæ
Marantaceæ
Najadaceæ
Palmaceæ
Pandanaceæ
Stemonaceæ
Triuridaceæ
Xyridaceæ
Zingiberaceæ

A thorough investigation of so many families requires much time and patience and the participation of a band of workers properly trained in embryological methods. Fortunately we have a number of qualified embryologists at Mysore, Bangalore, Benares, Annamalainagar, Poona, Madras, Agra, Meerut, Calcutta and other places, and I venture to hope that, with the co-operation of some of our colleagues in Europe and America, we may be able to prepare in this country a new "Comparative Embryology of Angiosperms" written more or less on the lines of Schnarf's great work (now 15 years old) in which each worker will write an exhaustive and critical account of the embryology of the particular group with which he is most familiar as the result of his *own* researches, for the literature on the subject is now too vast to be surveyed in a satisfactory manner by any one person. Students of wood structure like Record, Bailey and Wetmore, of chromosomology like Tischler, and floral anatomy like Eames and Arber are working towards the same end for their particular subjects. I expect that some of us present here will live to see the day when few families of flowering plants will need to be assigned by guess work, and in any case, whether the end comes sooner or later, we must travel hopefully towards it.

One fact to be noted in this connection is that although the embryologist cuts sections of the flower at various stages of development, he frequently confines his attention to the development and organisation of the embryo-sac and the subsequent changes which take place *inside* it or to a study of the meiotic divisions in the pollen mother-cells, while the structure of the anther and ovary wall, the placentation, the integuments, the nucellus, and the chalaza are either dealt with in a more or less cursory fashion or not described at all. This is unfortunate as all of these yield characters of great systematic value. The structure of the seed and the fruit receives even less attention probably because of the difficulty of sectioning them, but a judicious use of dilute hydrofluoric acid can sufficiently soften them in many cases without causing any appreciable harm to the tissues.

Last of all we come to the comparatively new science of experimental embryology. This is a more difficult field, but one which is full of promise in so many ways. As some one once said, the plant breeder puts pollen on the stigma and 'prays' for results in the ovary! For a scientific explanation of his successes and failures and for finding out the ways and means of increasing the former and remedying the latter, he must turn to the cytologist and the embryologist. The work that has been started in recent years on the effect of X-rays, heat, chemicals, etc., on the artificial induction of mutations is still in its infancy and it opens up vast possibilities before us. Here the breeder, the cytologist, the embryologist and the physiologist, must all join hands so that not only do we get the maximum results from what we have but we may evolve new and still better varieties of plants and thus add to the health and happiness of the world.

LITERATURE CITED

[In order that this list may not become unduly long, I have omitted from it a reference to such papers which have already been cited in the works numbered here as 16, 18, 24, 31, 33 and 37]

1. Buchholz, J. T. (1938) "Dissection, staining and mounting of the embryos of conifers," *Stain Tech.*, **13**, 53–64.
2. Cooper, G. O. (1942) "Microsporogenesis and development of seed in *Lobelia cardinalis*," *Bot. Gaz.*, **104**, 72–81.
3. Copeland, H. F. (1935) "The structure of the flower of *Pholisma arenarium*," *Amer. Jour. Bot.*, **22**, 366–83.
4. Darlington, C. D., and La Cour, L. (1942) *The Handling of Chromosomes*, George Allen & Unwin Ltd., London.
5. Ganesan, D. (1939) .. "Cytological studies in a chromosome ring-forming diploid *Notonia grandiflora* DC.," *Jour. Genetics*, **38**, 493–516.
6. Glišic', L. M. (1932) .. "Zur Entwicklungsgeschichte von *Lathræa squamaria* L.," *Bull. Inst. Jard. Bot. Univ. Beograd*, **2**, 20–56.
7. Gustafson, F. G. (1942) "Parthenocarpy : natural and artificial," *Bot. Rev.*, **8**, 599–654.
8. Heinricher, E. (1931) .. "Monographie der Gattung *Lathræa*," Jena.
9. Hillary, B. B. (1940) .. "Use of the Feulgen reaction in cytology II," *Bot. Gaz.*, **102**, 225–35.
10. Houk, W. G. (1938) .. "Endosperm and perisperm of coffee with notes on the morphology of the ovule and seed development," *Amer. Jour. Bot.*, **25**, 56–61.
11. Hutchinson, J. (1926, 1934) *The Families of Flowering Plants*, Vols. I and II, London.
12. Joshi, A. C. (1938) .. "A note on the morphology of the ovule of Rubiaceæ with special reference to cinchona and coffee," *Curr. Sci.*, **7**, 236–37.
13. Kausik, S. B. (1939) .. "Dissection and preparation of whole mounts of endosperm from the seeds of *Grevillea* (Proteaceæ)," *Stain Tech.*, **14**, 43–46.
14. Khan, Reayat (1943) .. "The ovule and embryo-sac of *Fouquieria*," *Proc. Nat. Inst. Sci. India*, **9**, 253–56.
15. Leitner, J. (1938) .. "Karminessigsäure als Hilfsmittel zur Untersuchung des Inhaltes reifer, volkommen ausgetrockneter Pollenkörner," *Zeitschr. wiss. Mikr.*, **55**, 48–50.
16. Maheshwari, P. (1937) "A critical review of the types of embryo-sacs in angiosperms," *New Phytol.*, **36**, 359–417.
17. ———— (1940) .. "The role of growth hormones in the production of seedless fruits," *Sci. & Culture*, **6**, 85–89.
18. ———— (1941) .. "Recent work on the types of embryo-sacs in Angiosperms : a critical review," *Jour. Ind. Bot. Soc.*, **20**, 229–61.
19. ———— (1942) .. "The embryo-sac of *Euphorbia heterophylla* L.—a reinvestigation," *Proc. Ind. Acad. Sci.*, B. **15**, 158–66.
20. ———— (1944*a*) "The origin of the haustoria in the ovule of *Lobelia*," *Jour. Ind. Bot. Soc.*, **23**, 78–81.
21. ———— (1944*b*) "The seed structure of *Ipomœa*, a criticism," *Sci. & Culture*, **9**, 557.
22. ——— and Singh, B. (1943) "Studies in the family Alismaceæ, V. The embryology of *Machærocarpus californicus* (Torr.) Small.," *Proc. Nat. Inst. Sci. India*, **9**, 311–22.

23. Maheshwari, P. and Srinivasan, A. R. (1944) "A contribution to the embryology of *Rudbeckia bicolor* Nutt.," *New Phytol.*, **43**, 135–42.

24. ——— and Wulff, H. D. (1937) "Recent advances in microtechnic I. Methods of studying the development of the male gametophyte of Angiosperms," *Stain Tech.*, **12**, 61–70.

25. Mendes, A. J. T. (1941) "Cytological observations on Coffea IV. Embryo and endosperm development in *Coffea arabica* L.," *Amer. Jour. Bot.*, **28**, 784–89.

26. Neumann, M. (1935) .. "Die Entwicklung des Pollen, der Samenanlage und des Embryosackes von *Pereskia amapola* var. *argentina*," *Oesterreich. bot. Zeitschr.*, **84**, 1–30.

27. Pannochia-Laj, F. (1938) "Embriologia e cariologia di *Lochnera rosea* L.," *Nuovo Giorn. Bot. Ital.*, **45**, 122–30.

28. Poddubnaja-Arnoldi, V. (1938) "A rapid method of embryological investigation," *Jour. Bot. U.R.S.S.*, **23**, 349–57.

29. Puri, V. (1941) .. "The life-history of *Moringa oleifera* Lamk.," *Jour. Ind. Bot. Soc.*, **20**, 263–84.

30. Sanchez, L. T. (1938) "Embryo-sac development in *Euphorbia heterophylla* Linn.," *Univ. Philippines Nat. & Appl. Sci. Bull.*, **6**, 59–75.

31. Schnarf, K. (1929) .. "Embryologie der Angiospermen," *Borntræger*, Berlin.

32. ——— (1929) .. "Die Embryologie der Liliaceæ und ihre systematische Bedeutung," *Sitzb. d. Akad. d. Wiss. Wien, math.-nat. Kl.*, **138**, Abt. I, 69.

33. ——— (1931) .. "Vergleichende Embryologie der Angiospermen," *Borntræger*, Berlin.

34. ——— (1933) .. "Die Bedeutung der embryologischen Forschung für das natürliche System der Pflanzen," *Biol. Gen.*, **9**, 271–88.

35. Tischler, G. (1910) .. "Untersuchungen ueber den Stärkegehalt des Pollen tropischer Gewächse," *Jahrb. wiss. Bot.*, **47**, 219–42.

36. Woodcock, E. F. (1943) "Seed development in the morning glory (*Ipomæa rubro-cærulea* Hook.)," *Papers Michigan Acad. Sci. Arts & Lett.*, **28**, 209–12.

37. Wulff, H. D., and Maheshwari, P. (1938) "The male gametophyte of angiosperms (a critical review)," *Jour. Ind. Bot. Soc.*, **17**, 117–40.

38. Wunderlich, R. (1937) "Zur vergleichenden Embryologie der Liliaceæ-Scilloideæ," *Flora N.F.*, **32**, 48–90.

CHROMOSOMES OF *ERYTHRINA INDICA* LAMK.

BY Y. SUNDAR RAO

Department of Botany, Benares Hindu University

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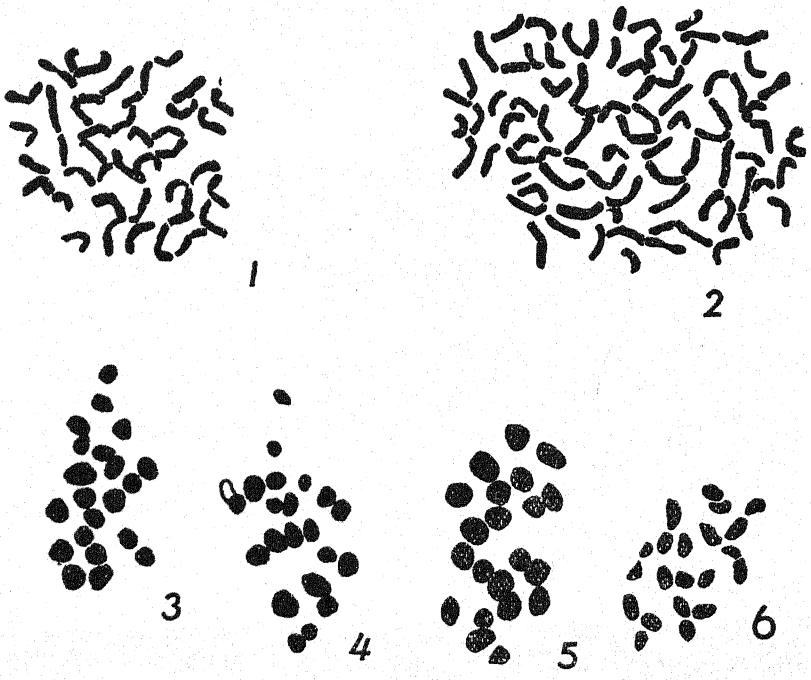
THE first observations on the chromosome numbers in the genus *Erythrina* L. (Fam. Papilionaceæ) were made by Tschechow and Kartaschowa, who reported for *Erythrina crista galli* L. [*Micropteryx crista galli* (L.) Walp.] in the same year in one of their papers (Tschechow and Kartaschowa, 1932*a*) *ca.* 40 and in another paper (Tschechow and Kartaschowa, 1932*b*) *ca.* 44 somatic chromosomes. The smallness of the chromosomes and their fairly large number in the root-tip cells might be the probable reasons for this obvious discrepancy. Next, Senn (1938) reported $2n = 42$ and $n = 21$ chromosomes in *Erythrina herbacea* L., and added that he too could not determine exactly the chromosome number in *E. crista galli*. The purpose of the present note is to report the chromosome number in the Indian Coral Tree, *Erythrina indica* Lamk., which grows wild along the Indian sea-coast and is widely planted in the gardens throughout the country for its large brilliant scarlet flowers. The materials for investigation, seeds and flowers, were obtained from trees growing at Guntur in the Province of Madras.

SOMATIC CHROMOSOMES

The somatic chromosomes were studied in root-tips obtained from germinating seeds. Most of the dividing nuclei showed 42 chromosomes (Fig. 1). These do not exhibit a wide range in size, but are small, slender objects, of nearly the same size and show either median or submedian attachment constriction. The somatic karyotype of *Erythrina indica* Lamk. thus appears to be identical with that of *E. herbacea* L. as sketched by Senn (1938).

During the examination of the sections of the root-tips, besides the monosomatic cells, some disomatic cells were also observed. The dividing nuclei of such cells showed 84 chromosomes (Fig. 2). Such cases of somatic doubling of chromosomes have been reported already in many Leguminosæ. Senn (1938) in his extensive work on the cytology of this family found tetraploid cells in *Albizzia Julibrissin* and *Cassia nictitans*, and Iyengar (1938) in *Cicer arietinum*, but the most comprehensive observations in this respect have been made by Wipf (1939) and Wipf and Cooper (1938 and 1940). They have reported the general occurrence of cells with tetraploid nuclei in the roots of several Leguminosæ, such as *Pisum sativum*, *Lathyrus latifolius*, *L. odoratus*, *Lespedeza tomentosa* and *Vicia villosa*, and find a definite



Figs. 1–6. *Erythrina indica* Lamk.—Fig. 1, Somatic metaphase showing 42 chromosomes. Fig. 2. Same showing a tetraploid nucleus with 84 chromosomes. Figs. 3–5. Polar views of Metaphase I ($n = 21$). Fig. 6. Metaphase II; only one plate of a P.M.C. is shown; $n = 21$. ×3,000.

relationship between the normal occurrence of disomatic cells in the roots of these plants and the formation of root nodules. The genetic significance of somatic doubling of chromosomes in restoring the fertility of sterile hybrids and in the origin of new species is already well known and need not be mentioned again here.

MEIOSIS

Observations on pollen mother cells undergoing meiosis showed $n = 21$ both at the I and II metaphase (Figs. 3–6). There are slight differences in size among the various bivalents at the I metaphase, but much importance need not be attached to this, as the size of bivalents in the polar views is determined by the presence and number of chiasmata (*cf.* Upcott, 1936). Polar views of meiotic chromosomes in *Erythrina indica* are also characterised by a marked degree of secondary association (Figs. 3 and 4). Groups of 2, 3 and 4 bivalents are quite common during the I metaphase and secondary association persists even in II metaphase.

DISCUSSION

The following table summarises the chromosome numbers reported so far in the genus *Erythrina* L.:—

Chromosome Numbers in Erythrina *L.*

Species	*n*	2 *n*	Author
E. crista galli L.		*ca.* 40	Tschechow and Kartaschowa (1932*a*)
Do.		*ca.* 44	Do. (1932*b*)
E. herbacea L.	21	42	Senn (1938)
E. indica Lamk.	21	42	This paper

The occurrence of $n = 21$ and $2n = 42$ chromosomes both in *E. herbacea* and *E. indica* make it very probable that in *E. crista galli* also there are 42 somatic chromosomes.

The occurrence of a rather high chromosome number in species of *Erythrina* as compared with most of the Papilionaceæ and secondary association both during the I and II meiotic divisions suggests that the genus *Erythrina* is of a polyploid nature. Taking into account that $n = 21$ is an unusual chromosome number in the Papilionaceæ, Senn (1938) remarked that this may indicate an ancestry through a 7 series or be the result of hybridisation from $n = 10$ and $n = 11$ ancestors with subsequent amphidiploidy. The latter suggestion, according to him, appears possible in view of the taxonomic position of the genus between forms with a basic number 10 and forms with a basic number 11. Future investigators of the cytology of *Erythrina* have to study this problem.

In the end, the author desires to express his appreciation to Dr. A. C. Joshi for his kind interest in the investigation and help in the preparation of this note.

LITERATURE CITED

Iyengar, N. K. (1939) .. "Cytological investigations on the genus *Cicer*," *Ann. Bot., New Ser.*, **3**. 271-306.

Senn, H. A. (1938) .. "Chromosome number relationships in the Leguminosæ", *Bibliog. Genet.*, **12**, 175–336.

Tschechow, W. and Kartaschowa, N. (1932*a*) "Karyologisch-systematische Untersuchung der Tribus Loteæ und Phaseoleæ Unterfam. Papilionatæ," *Cytologia*, **32**, 221–49.

———— (1932*b*) .. "Karyo-systematische Untersuchung der Tribus Loteæ Benth. und Phaseoleæ Bronn. Fam. Leguminosæ," *Bull. Toms. State Univ.*, **85**, 1–22 (Quoted by Senn, 1938).

Upcott, M. (1936) .. "The parents and progeny of *Aesculus carnea*," *Jour. Genet.*, **33**, 135–149.

Wipf, L. (1939) .. "Chromosome numbers in root nodules and root tips of certain Leguminosæ," *Bot. Gaz.*, **101**, 51-67.

——— and Cooper, D. C. (1938) "Chromosome numbers in nodules and roots of red clover, common vetch and garden pea," *Proc. Nat. Acad. Sci. Wash.*, **24**, 87–91.

———— (1940) .. "Somatic doubling of chromosomes and nodular infection in certain Leguminosæ," *Amer. Jour. Bot.*, **27**, 821–24.

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EMBRYOLOGICAL STUDIES IN THE THYMELÆACEÆ

I. *Thymelaea arvensis* Lamk.

BY J. VENKATESWARLU

Andhra University

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THYMELÆACEÆ, by itself a very natural group, shows no close affinities with other families of flowering plants. It has been investigated by embryologists from time to time, but these investigations are mainly concerned with the development of pollen, ovule and embryo-sac. A few observations have been made also on the development of the embryo, but these accounts are very fragmentary and do not give either a correct or a complete picture of embryo development in any species. This has led the author to take up the present work.

PREVIOUS WORK

Broniart (1826) was the earliest botanist to pay attention to the embryology of Thymelæaceæ. He recognised the conducting function of the tissue arising at the base of the style (now called the obturator). Next, Hofmeister (1849) studied *Daphne laureola*, in which he observed a normal type of embryo-sac development. He also recorded that the mature embryo-sac was already formed before the onset of winter. Beauregard (1877) investigated the structure and development of the fruit in *Daphne*. Capus (1878), in his studies on the anatomy of conducting tissue in the angiosperms, drew attention to the formation of a bundle of tissue traversing from the base of the style to the micropyle. Vesque (1879) made some observations on *Daphne mezereum*. According to him, the archesporium in the ovule consists of a single cell and the embryo-sac development follows the normal type. The formation of nucellar cap and obturator was also studied by him. Prohaska (1883) studied the development of the embryo-sac and the endosperm in *Daphne*. He reported the occurrence of many antipodals in *Daphne Blagayana*. Schimper (1885) is referred to by Schnarf (1929)

as having reported the occurrence of plastids in the egg of the above species.

Strasburger (1884, 1885, 1909 and 1910) studied the embryology of *Daphne*, *Gnidia* and *Wikstræmia* and particularly the problem of parthenogenesis in *Wikstræmia indica*. He noted unicellular archesporium in the ovule, a normal type of embryo-sac development, many antipodals in the embryo-sac of *Daphne Blagayana* and only three in *Daphne alpina* and *Wikstræmia indica*, and the formation of the nucellar cap in the ovule. The development of the obturator and the endosperm in *Daphne* was also studied by him. He also made some observations on the structure and development of the anther and pollen. He states that the tapetal nuclei divide mitotically in *Wikstræmia indica*.

Winkler (1904, 1906) made an embryological study of *Wikstræmia indica* and investigated the problem of parthenogenesis in it. He traced the structure and development of the anther and pollen. According to him, the tapetum is formed by the innermost wall layer of the anther and its cells contained 2–6 nuclei. He described a normal structure in the embryo-sac. He studied the formation of obturator and pointed out that it formed a plug in the micropyle in *Wikstræmia indica*. He also made some observations on the development of the endosperm and embryo.

Osawa (1913) investigated the development of pollen and embryo-sac of *Daphne* with special reference to sterility in *D. odora*. According to him, the division in the pollenmother cell is simultaneous. He reported the 3-nucleate condition of the mature pollen, and also some irregularities in the pollen formation in *D. odora*, besides degenerations in pollen and embryo-sacs. According to him, a linear tetrad of megaspores is usually formed in *D. odora*, but he also observed occasional formation of a T-shaped tetrad. Normally the chalazal megaspore is the functional one. He, however, found a few exceptional cases in *D. odora*, where a megaspore other than the chalazal one of the tetrad was functional. He noted 3–6 antipodals in the embryo-sac in *D. odora* and 30 or more in *D. pseudo-mezereum* and *D. koiusiana*.

Guèrin (1913, 1915) made a comprehensive study of the structure and development of the ovule and seed in Thymelæaceæ. He found vascular strands in the nucellus of *Dicranolepis*, *Craterosiphon* and *Synaptolepis*. His observations on the structure and development of the embryo-sac agree with those made earlier by others. He reported the occurrence of two cases of bilateral tetrads of megaspores in *Daphnopsis Schwartzii*. Guérin also reported more than three antipodals in *D. Schwartzii* and many in *Thymelæa passerina* and *Dirca palustris*. He made some observations on the endosperm. Structure of the seed-coat was also described.

Dahlgren (1915) in his studies on the development of pollen, ovule and seed in angiosperms, mentions the formation of normal type of embryo-sac in *D. mezereum* before the advent of winter. Yamaha

(1926), in his comprehensive study of cytokinesis in the formation of pollen tetrads in the various plant groups, states that the cytokinesis in *Daphne* takes place by furrowing and passes through very rapidly. He also refers to the formation of a transitory cell plate after the heterotypic division in the pollen mother cells. Joshi (1937) noted 3-nucleate condition in the mature pollen of *Wikstræmia indica*, *Thymelæa arvensis* and *Daphne mezereum*.

Fuchs (1938) gave a detailed account of the embryology of *Daphne odora* and made some observations on the embryology of *D. cneorum* and *Passerina pectinata*. She made a comparative study of the structure of pollen in *Daphne odora*, *D. cneorum*, *D. Blagayana*, *D. mezereum*, *Passernia pectinata*, *P. filiformis*, *Pimelea decussata*, *Pimelea ligustrina* and *P. spectabilis*. In all these she finds the mature pollen to be 3-nucleate and sperm cells to be elongated in the form of spirally twisted bands. She also describes the presence of rods and spines as structural features of the exine. The pollen has many germ pores. In *D. laureola*, *D. cneorum* and *Passerina pectinata*, she notes the formation of a nucellar cap, the presence of a plate-shaped tissue in the chalaza of the ovule and a conducting strand of elongated cells connecting it with the antipodal end of the embryo-sac. She also gives an account of the structure of the obturator. The embryo-sac development in the species studied follows the normal type. She reports the occurrence of linear, T-shaped and bilateral tetrads of megaspores. She finds 30–40 antipodals in the embryo-sac of *Daphne laureola*, *D. cneorum* and *Passerina pectinata*. According to her, these persist as dark points in ripe seeds. She states that polar nuclei lie close together near about the chalazal end and probably fuse just before fertilisation. The same was observed earlier by Winkler (1906), Strasburger (1909) and Guérin (1915). She followed the endosperm development in *D. laureola*. The fusion nucleus, according to her, divides before the fertilised egg. In the later stages cell formation was also noted by her. She also made a few observations on the embryo development in *D. laureola* and described the seed-coat structure.

Mauritzon (1939) described the structure and development of the ovule and seed in *Phaleria capitata*. He observed a remarkably extensive growth of the chalazal part in the ovule. He also made a few observations on the development of the pollen and embryo-sac in the same species.

Kausik (1940) published an account of the structure and development of the embryo-sac in *Lasiosiphon eriocephalus*. He described the structure of the anther and traced the origin of the tapetum. He noted the formation of a nucellar cap and conducting strand in the nucellus below the antipodal end of the embryo-sac. The development of the embryo-sac is normal. He noted the occasional presence of a spherical body in the synergids. According to him, the endosperm is of the free nuclear type. In the early stages the endosperm nuclei show a paired arrangement. He described a dense mass of cytoplasm in the chalazal part of the embryo-sac and states that the endosperm nuclei are large in this region and lie embedded in the plasma accumulated there.

He also made a few observations on the embryo. A 3-celled proembryo is formed. Further development is not followed in detail, but he states that a spherical embryo is formed after a few divisions. He also described the seed-coat structure, which is in agreement with the earlier accounts.

Material and Methods

The present paper deals with the development of the anther, pollen, ovule, embryo-sac, endosperm, embryo and seed in *Thymelæa arvensis* Lamk. This species grows in the upper Punjab, Kashmir, N.W.F.P., and extends from Afghanistan westwards to France and North Africa.

The material used in this investigation was very kindly placed at my disposal by Dr. A. C. Joshi of the Benares Hindu University along with a few prepared slides. It was collected from plants growing at Sopore (Kashmir), in the month of June 1938 and was fixed in formalin-acetic-alcohol. The customary methods of dehydration and infiltration were followed. Sections were cut 10–14 μ in thickness and were stained with Heidenhain's iron-alum-hæmatoxylin. Tuan's method of destaining with picric acid was followed.

Organogeny of the Flower

The flowers arise singly in the axil of leaves. The floral parts arise in acropetal succession, the perianth making its appearance first, followed by the stamens and, last of all, the gynœcium (Figs. 14 *a*, *b* and *c*). The single whorl of perianth is urceolate and its 4 lobes are imbricate in bud. The upper part of the perianth shrivels up in the fruiting stage and the whole perianth remains as a membranous covering round the ovoid fruit.

The stamens are 8 in number (in two whorls) and are adnate to the perianth. The ovary is raised on a short stalk and contains a laterally attached single ovule. The style is short and ends in a stigma the surface cells of which form a number of papillæ. A prominent annular disc-scale develops round the base of the ovary.

Development of the Anther and Pollen

The primary archesporium in the anther consists of only one row of cells in each of the four anther-lobes (Figs. 1 and 2). Soon after their differentiation the primary archesporial cells undergo a periclinal division, forming a layer of primary parietal cells towards the outside and a layer of primary sporogenous cells towards the inside (Fig. 3). The parietal layer of cells again undergoes another periclinal division and forms two layers of wall cells below the epidermis (Fig. 4). The inner of these only divides further into two layers, thus ultimately forming three wall layers below the epidermis (Fig. 5). The layer of wall cells immediately beneath the epidermis forms the endothecium, while the one immediately outside the sporogenous cells forms the tapetum. It is of the secretory type.

The tapetal nuclei divide mitotically and the tapetal cells become two-nucleate about the time when the pollen mother cell nuclei are in

the prophase of I meiotic division. The two nuclei lie closely appressed to each other. In the final stages the tapetal cell nuclei are found to contain up to 4 nucleoli. The tapetal cells, before degeneration, become filled with very small vacuoles.

In the mature anther, due to the growth of the pollen mother cells and the tapetum, the wall layer between the endothecium and the tapetum gets crushed. With the further growth of the anther, the epidermis gets very much stretched, the endothecium further enlarges and the tapetum slowly degenerates. In very mature anthers the epidermis is so thinned out that it is hardly perceptible as a separate layer at some places (Fig. 6).

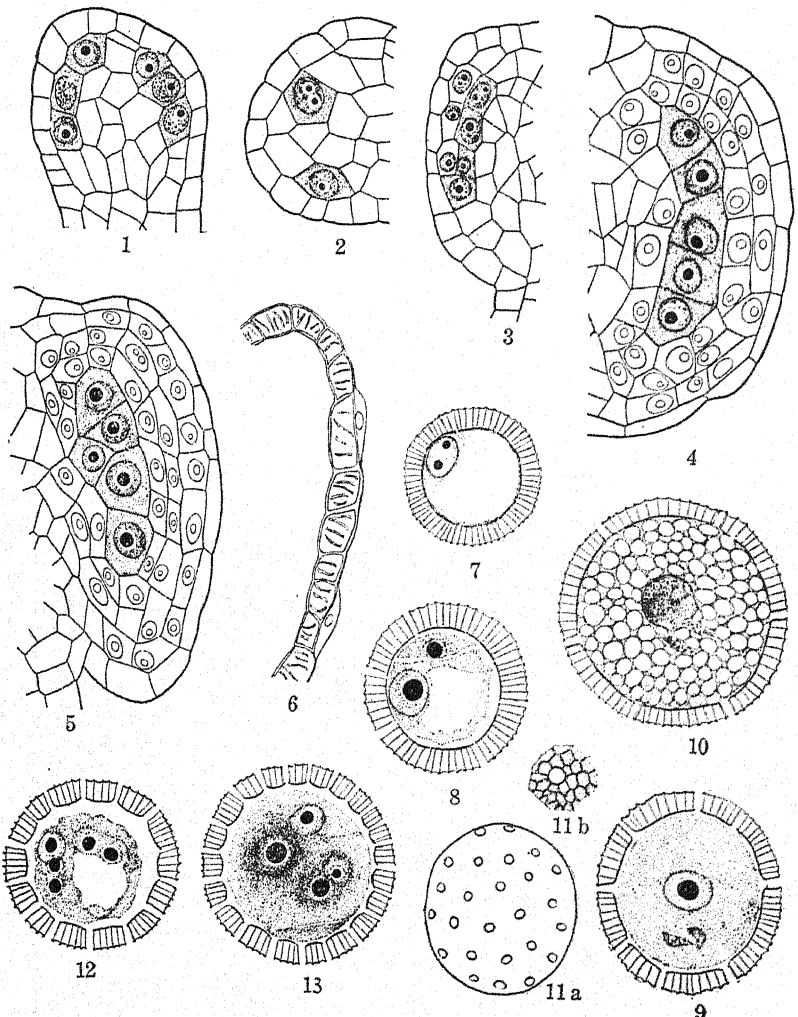
Usually the primary sporogenous cells enlarge and form the pollen mother cells. However, quite frequently, a cell or two of the primary sporogenous layer may divide once before forming pollen mother cells. The pollen mother cell nuclei undergo two meiotic divisions and form pollen tetrads ultimately. During meiosis they do not round off but remain packed together within the mother cell walls. The pollen mother cells divide simultaneously. The orientation of spindles at the II meiotic division has been found to be at right angles to each other, but sometimes parallel arrangement is also seen. This results in the formation of both tetrahedral as well as bilateral pollen tetrads. Cytokinesis appears to pass through rapidly and takes place by furrowing.

The exine and intine are formed before the pollen grain becomes two-nucleate. Fully formed uni-nucleate pollen grain has a large vacuole and the nucleus occupies a peripheral position. Usually, it is 1-nucleolate, but two nucleoli have been observed quite frequently (Fig. 7). The nucleus divides and forms two nuclei which show considerable difference in their size. The larger one is the vegetative nucleus and the smaller is the generative nucleus. The latter is organised into a definite lenticular cell separated from the vegetative one by a curved wall (Fig. 8). This wall, however, soon disappears, both the nuclei being left in the general cytoplasm of the pollen grain. The formation of such a lenticular cell separated from the vegetative one by an evanescent wall was also noted in some Thymelæaceæ by Fuchs (1938).

The generative nucleus divides into two male nuclei which finally form two elongated sperms. The pollen grain thus becomes 3-nucleate, which condition has been noted as characteristic feature of the pollen in Thymelæaceæ (Osawa, 1913 ; Joshi, 1937 ; Fuchs, 1938). The pollen grain shows a general increase in size and in mature condition gets filled with starch grains (Fig. 10). The sperms are elongated and spindle-shaped. The chromatin is unevenly distributed and the sperms look like spirally twisted bands. The same was also observed by Fuchs (1938) in *Daphne*. The tube nucleus is spherical in the early stages, but later it takes an irregular shape and stains very deeply as in Amarantaceæ (Kajale, 1940).

The exine and intine are formed in the uni-nucleate stage of the pollen grain. The former is much thicker than the intine. As seen

in sections, it is composed of light and dark staining parts, the latter taking the form of radially arranged rods covered on all sides by the light staining portions of the exine (Figs. 7–10, 12 and 13). The exine protrudes a little outwards on the surface where the rod-like portions are situated giving rise to small spines. The latter are united



Figs. 1–13. *Thymelæa arvensis.*—Figs. 1–5. Various stages in the development of the anther. Fig. 2. Shows a transverse section, the rest represent longitudinal sections. Fig. 6. L.S. of wall of a mature anther. Figs. 7–10. Pollen grains in various stages of development. Fig. 11 *a*. Surface view of pollen grain showing germ pores. Fig. 11 *b*. A portion of exine showing a germ pore and sculpture on the surface. Figs. 12 and 13. Abnormal pollen grains showing extra nuclei. Figs. 1–5, ×546; Fig. 6, ×263; Figs. 7–11 *a* and 12–13, ×833; Fig. 11 *b*, ×3,360.

on the surface by ridges giving rise to a network like pattern on the outer surface of the exine (Fig. 11 *b*). The meshes of the network are usually 6-sided. There are many germ pores (about 50) and they are approximately equally spaced. They are usually circular in outline. Their arrangement on the exine is rather interesting. Usually, each pore, taken as centre, is surrounded by six other pores (Fig. 11 *a*). The pore membrane does not protrude out of the germ pores.

The mature pollen is sphæroidal in shape and the exine gets thinner on account of the stretching of the wall due to the growth of the protoplast of the pollen grain, very much like what has been described by Kajale (1940) in some Amarantaceæ. The diameter of the mature pollen grain approximately measures 35 μ–37 μ and that of the germ pore 1·6 μ–1·8 μ. The distance between two germ pores (centre to centre) is about 5–6 μ.

Abnormal Pollen

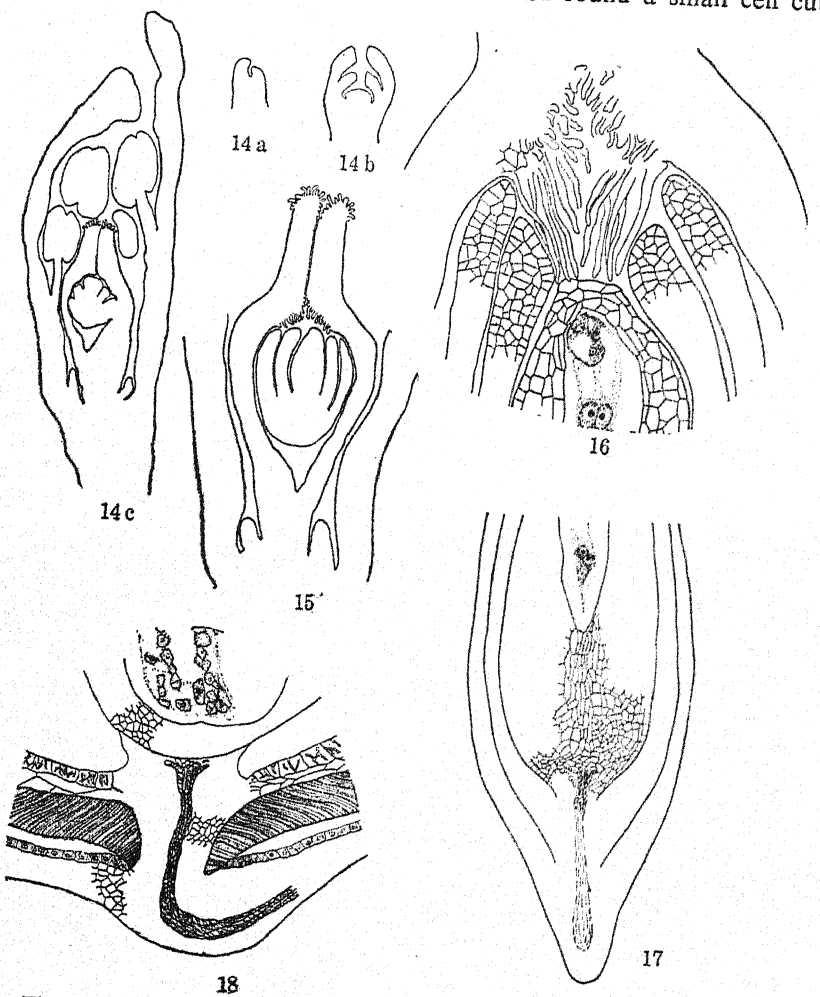
The pollen grains, as stated above, are usually 3-nucleate at the shedding stage. When the generative nucleus divides, it forms two sperm nuclei which are round in shape in the beginning. The vegetative nucleus is bigger in size and possesses a nucleolus. It stains less deeply than the generative nucleus with iron-alum-hæmatoxylin. The latter is distinguished from the vegetative nucleus by the absence of a distinct nucleolus, smaller size and deep staining capacity with iron-alum-hæmatoxylin. These features are also shared by the two sperm nuclei that arise from it.

Two exceptional cases of multi-nucleate pollen have been encountered in my preparations (Figs. 12 and 13). Fig. 12 shows a pollen grain with 5 nuclei, out of which one is larger in size. It shows a distinct nucleolus and stains less deeply than the rest with iron-alum-hæmatoxylin. The rest of the four are much smaller in size, take a deep stain and show no distinct nucleolus. From these features, it appears that the two sperm nuclei have undergone an extra division producing four daughter nuclei resembling the sperm nuclei in their general appearance. It may be mentioned here that four male nuclei (of about equal size), in pairs, have been observed by Dutt and Subba Rao (1933) in some pollen grains and in a pollen tube that has just reached the embryo-sac in the sugarcane (cross vellai ♀ ×B. 3412 ♂). Fig. 13 shows a pollen grain with four nuclei, out of which two are larger in size, stain less deeply and show a nucleolus, while the rest of the two resemble sperm nuclei. In this case, it appears that the extra nucleus has arisen due to the division of the vegetative nucleus.

Wulff and Maheshwari (1938) list a number of such abnormal cases of pollen having more than three nuclei in their review of the male gametophyte of angiosperms. The list includes *Lilium tigrinum* (Chamberlain, 1897), *Eicchornia crassipes* (Smith, 1898), *Sparganium simplex* (Campbell, 1899), *Yucca recurva* (Woycicki, 1911), *Cuscuta epithymum* (Federtschuk, 1931), *Atriplex hymenolytra* (Billings, 1934) and *Stelleria media* (Joshi, P. C., 1936). Bhargava (1936) reports

a 4-nucleate condition in many pollen grains of *Chenopodium album*. Juliano and Alcala (1935) reported the occurrence of 2–7 vegetative nuclei in *Musa errans* (Blance) Teodore var. *Botoan* Teodore.

Citing some of the above cases, Coulter and Chamberlain (1903) write, "In *L. tigrinum*, Chamberlain has often found a small cell cut



Figs. 14–18. *Thymelæa arvensis*.—Figs. 14 *a*, *b*, *c*. Stages in the development of the flower. Fig. 15. L.S. of the gynœcium showing the development of the obturator, ovule and the disc scale at the base of the stalked ovary. Fig. 16. L.S. base of the style and upper part of a mature ovule showing the obturator in the funnel-shaped micropyle. Fig. 17. L.S. of ovule (except the micropylar part) showing the conducting strand connecting the antipodal end of the embryo-sac with the ovular vascular trace. Fig. 18. L.S. of the chalazal end of an old ovule. Figs. 14 *a*, *b* and *c*, ×185; Fig. 15, ×78; Fig. 16, ×213; Figs. 17 and 18, ×126.

off by the microspore before the appearance of the tube and generative nuclei and the same cell was noted after the division of the generative nucleus. A similar cell was found by Smith in *Eicchornia crassipes* and by Campbell in *Sparganium simplex*. It is suggestive of true vegetative or prothallial cell, two of which so commonly occur in gymnosperms but the phenomenon is too unique as yet among the angiosperms to deserve more than a mention." According to Wulff and Maheshwari, Billings (1934) does not think it improbable that "an angiosperm would now and then be found exhibiting an atavistic tendency in producing a prothallial cell". P. C. Joshi (1936), however, thinks that there is no scope for interpreting the extra nucleus as a prothallial cell in the exceptional case of the 4-nucleate pollen grain recorded by him in *Stelleria media*. From the two cases recorded by the writer, it appears that the extra nuclei may be formed due to the further division of either the vegetative nucleus or the generative one.

Development and Structure of the Ovule and Embryo-sac

Ovule.—The ovary contains a single anatropous ovule. It is laterally attached, with the micropyle pointing upwards. First, it appears as a small hump. At about the time of division of the primary archesporial cell into an outer parietal cell and an inner megaspore mother cell, the integuments start as two annular rings one below the other. Ultimately the fully developed ovule assumes the anatropous form. In the early stages of development there is some space below the ovule (Figs. 14*c* and 15), but afterwards it is all occupied and the ovule touches the lower end of the ovary. There is no suggestion of the presence of a second ovule.

The ovule has two integuments. The micropyle, in fertilisable ovules, is funnel-shaped. It is formed by both the integuments, though the inner takes greater part at this stage. The micropyle receives the obturator. After fertilisation, the obturator dwindles gradually until, finally, it disappears in the seed. The integuments also grow and come close together forming a narrow micropyle in the seed. To start with the integuments are 3 cells in thickness, but in later stages the outer one becomes 4 cells in thickness and the inner 5 cells in thickness. Their various cell layers undergo different changes in the seed-coat. These are described later.

The nucellus is fairly massive. The nucellar epidermis, in the micropylar region, shows periclinal divisions and forms a nucellar cap 2–3 cells in thickness (Figs. 21, 22 and 23). In the mature ovules, the nucellus above the micropylar part of the embryo-sac is about 4–5 cells in thickness (including the cells of the nucellar cap) and, at the apex forms a slightly elongated protrusion. Usually it is about 4 cells in thickness on the sides of the embryo-sac and 15–20 cells in thickness below the antipodal end of the embryo-sac. In the chalazal region is developed a strand of elongated cells connecting the vascular bundle of the funicle and the antipodal end of the embryo-sac (Fig. 17). Such a conducting strand in the chalazal region of the ovule was also noted by Fuchs (1938) in some members of Thymelæaceæ and by

Kausik (1940) in *Lasiosiphon eriocephalus*. Guérin (1913) described the presence of vessels in the nucellus of *Dicranolepis*, *Craterosiphon* and *Synaptolepis*. Such vessels, however, are absent in *Thymelæa arvensis*, which, in this respect, resembles *D. laureola*, *D. cneorum*, *Passerina pectinata* (Fuchs, 1938) and *Lasiosiphon eriocephalus* (Kausik, 1940). A similar conducting strand in the ovule is also found in Lythraceæ (Joshi and Venkateswarlu, 1935 *a*, 1935 *b*, 1936; Venkateswarlu, 1937 *a*), in *Duabanga sonneratioides* (Venkateswarlu, 1937 *b*) and in Geissolomataceæ (Stephens, 1909).

Megasporogenesis and Embryo-sac.—The primary archesporium in the ovule consists of a single cell, which differentiates much before the integumental primordia appear (Fig. 19). It undergoes a periclinal division giving rise to an outer cover cell and an inner megaspore mother cell. Even before this division, the cells of the epidermis have divided once or twice periclinally to form the nucellar cap. The primary parietal cell undergoes a periclinal division and ultimately gives rise to two layers of parietal tissue under the nucellar cap. In *Daphne alpina* (Strasburger, 1909), *D. mezereum* (Vesque, 1879), *D. odora* (Osawa, 1913), *D. laureola*, *D. cneorum* and *Passernia pectinata* (Fuchs, 1938), a more extensive parietal tissue is formed making the megaspore mother cell deep-seated.

The megaspore mother cell forms a linear tetrad of megaspores, the chalazal-most of which is the functional one (Figs. 21–22). The three micropylar megaspores degenerate and they can be seen at the 4-nucleate stage of the embryo-sac (Fig. 23). No cases of T-shaped or bilateral tetrads of megaspores have been met with as in *D. alpina* (Strasburger, 1909), *D. odora* (Osawa, 1913), *D. Schwartzii* (Guérin, 1915) and *D. laureola* (Fuchs, 1938).

The development of the embryo-sac is according to the normal type (Figs. 21–27). The binucleate embryo-sac is characterised by the persistence of the chalazal vacuole. The same feature has been observed in *Lasiosiphon eriocephalus* (Kausik, 1940) and in Lythraceæ (Joshi and Venkateswarlu, 1935 *a*, 1935 *b*, 1936). In the 8-nucleate embryo-sac, the organization of the antipodals takes place slightly earlier than the egg-apparatus (Fig. 24). The two polar nuclei move towards the centre and meet about the middle of the embryo-sac. Usually the polar nuclei are 1-nucleolate, but in one case each of them has been found to be 2-nucleolate (Fig. 25). They fuse just before triple fusion. The two synergids, when fully developed, are hooked and have the usual chalazal vacuole. There is also seen a small vacuole in the apical region of the synergid (Fig. 28). The egg is usually situated a bit deeper than the synergids (Fig. 27) and has the usual flask-shaped form with a large vacuole above the nucleus (Fig. 29). In the early stages of the 8-nucleate embryo-sac, the antipodals are formed into three cells. Later, they multiply and form a large number of small cells (Figs. 27 and 31). Usually about 25–30 cells may be counted. During the endosperm formation, the antipodal end of the embryo-sac elongates and leaves behind the antipodal mass of cells on one side at about the middle of the embryo-sac (Figs. 32 and 33).

As can be seen from the review of previous work, many antipodals have been reported previously in *Daphne Blagayana, D. pseudo-mezereum, D. koiusiana, D. laureola, D. cneorum, Passerina pectinata, Dirca palustris* and *Thymelæa passerina.*

The form of the embryo-sac varies at the various stages of its development (Figs. 24–29 and 30–34). Up to the time of fertilisation it is contained in the upper half of the nucellus, but later it elongates and extends throughout the length of the nucellus. With the growth of the embryo-sac the surrounding nucellar tissue is crushed.

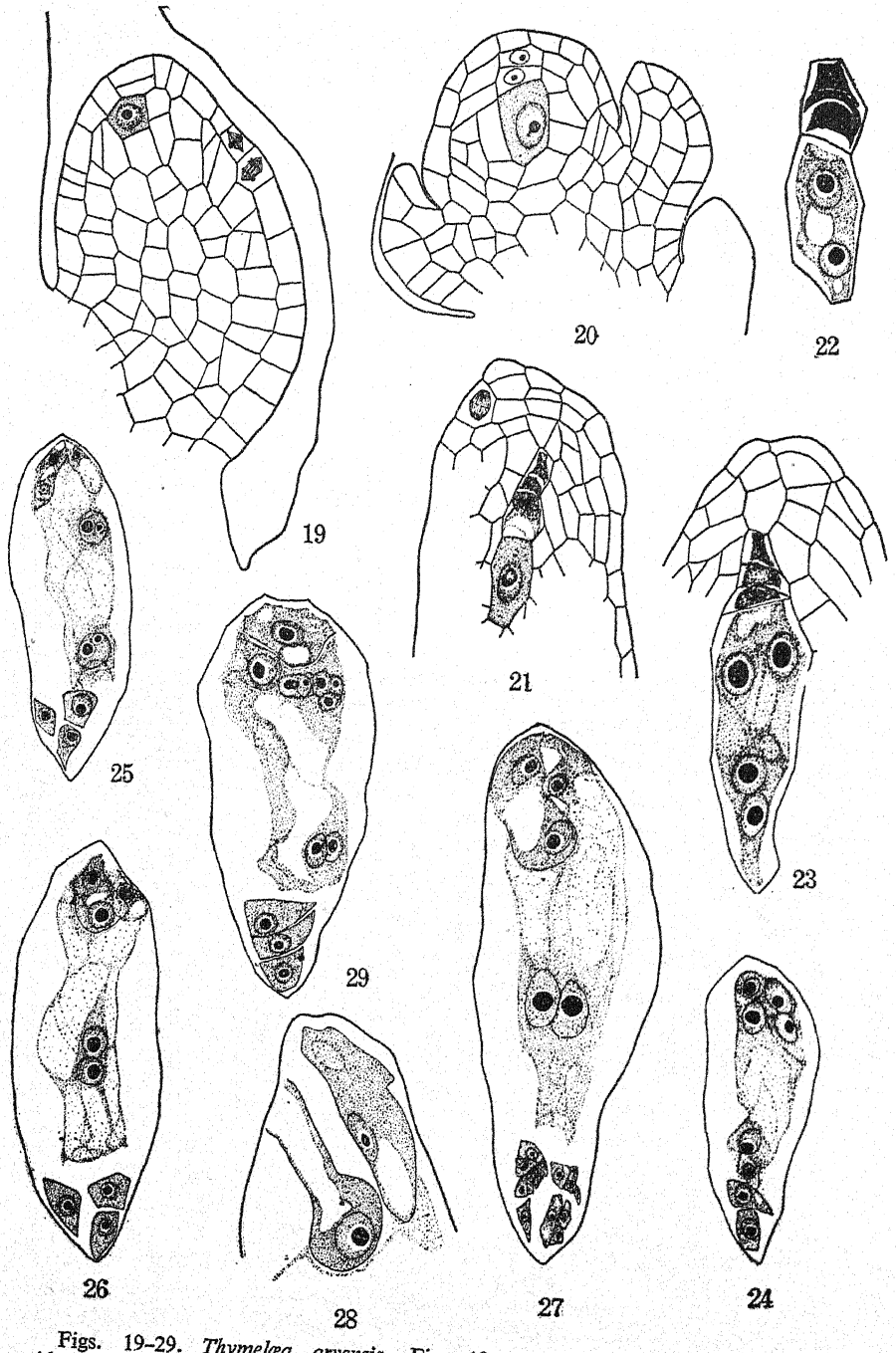
An Abnormal Embryo-sac

As stated above, the embryo-sac in *Thymelæa arvensis* is formed according to the normal type. The mature embryo-sac contains very small antipodals in addition to the egg apparatus and the two polar nuclei. A single case of an abnormal embryo-sac with more than the usual number of nuclei has been met with. Fig. 29 shows the same. It is in an early stage of development and shows three antipodal cells and two polar nuclei lying together near the antipodal end, while in the micropylar end are seen two cells and five nuclei. Out of the two cells, one can be recognised as the egg judged from its form and structure. The second cell has a distinct vacuole in the basal part below its nucleus and seems to be the only synergid formed. Out of the five nuclei, three are found together in close association and indeed in the final stages of separation from each other. From their arrangement and association they, undoubtedly, seem to have arisen from the same nucleus in an amitotic manner. Out of the remaining two nuclei, the nucleolus of one is constricted and it seems to be on its way to divide amitotically. The situation seems to have arisen in the following manner :—First of all, as usual, two polar groups of four nuclei each should have been formed. Soon after, the antipodals are organised and the polar nuclei move towards the centre, meet each other and finally take their position together near the antipodal end. At about the same time, the egg cell and one synergid are differentiated at the micropylar end, out of the three nuclei left over there. The undifferentiated nucleus undergoes an amitotic division and then one of the two daughter nuclei, in its turn, follows suit. Thus, it ultimately leads to the formation of extra nuclei observed in the case recorded here.

Abnormalities in the structure of the embryo-sac have been previously noted in other flowering plants. They may be grouped as below :

(i) *Embryo-sacs that show fewer than* 8 *nuclei.*—These arise either due to the suppression of one or more divisions or degeneration of a few of the 8-nuclei formed (usually in the chalazal pole of the embryo-sac). Instances of this kind are noted in good many plants and *Oenothera* (4-nucleate) type is supposed to be formed from a total suppression of development of the chalazal group.

(ii) *Embryo-sacs that show* 8 *nuclei but with abnormal organization of their constituents.*—The abnormal organization of the constituents may involve loss of polarity, reversed polarity, want of differentiation



Figs. 19-29. *Thymelæa arvensis*.—Fig. 19. Primary archesporium. The epidermis of the ovule shows a periclinally divided cell. Fig. 20. M.M. cell

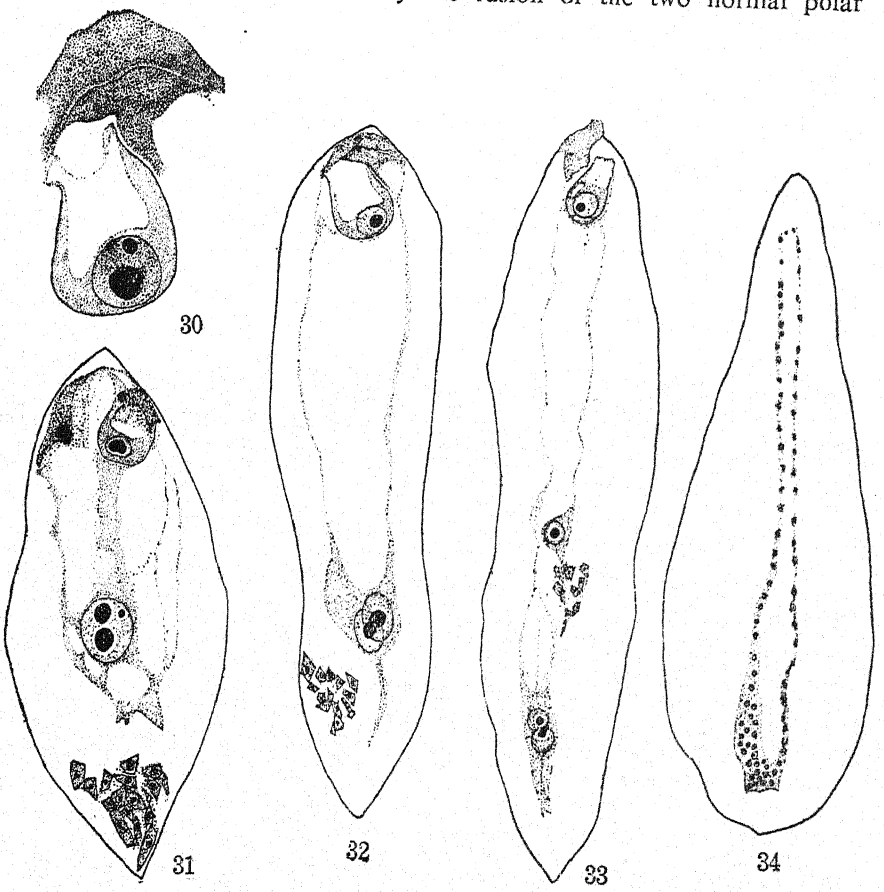
with two parietal cells above it. Fig. 21. L.S. of the nucellus of an ovule showing a linear tetrad of megaspores and nucellar cap formation. Fig. 22. 2-Nucleate embryo-sac. Figs. 23–27. Various stages in the embryo-sac development. Fig. 27. Shows a mature embryo-sac with many antipodals. Fig. 28. L.S. micropylar end of the embryo-sac showing the egg and a synergid. Fig. 29. An abnormal embryo-sac with extra nuclei. See text for further explanation. Figs. 19–21, 24–27 and 29, ×560; Figs. 22, 23 and 28, ×820.

in the constituents of the various groups, reduction in the number of members of one group and consequent increase in one or both the remaining groups, formation of extra egg cells at the expense of synergids, formation of extra number of synergids and eggs at the expense of other constituents, lack of differentiation in one or more constituents of either polar group, etc. Cases falling in this category are rare and always bear the character of abnormality.

(iii) *Embryo-sac with more than* 8 *nuclei.*—Extra nuclei arise due to secondary increase in the number of nuclei in either polar group. The extra nuclei so formed may add to the usual number constituting the egg-apparatus or polar nuclei or the antipodals. Secondary increase in the number of antipodals is found to occur as a normal feature in the embryo-sac of some plants, but secondary multiplication and increase in the micropylar group is very rare and always bears the character of abnormality.

The abnormal case of an embryo-sac in *Thymelæa arvensis* described above belongs to the last group. Schnarf (1929) and later Maheshwari (1941) enumerated important cases of abnormalities in the embryo-sac. Among these are included those that show extra nuclei. In 1880, Mellink recorded in *Luffa petiolata*, an embryo-sac divided into many cells and without any differentiation of either the antipodals or the egg-apparatus. In 1905, Shattuck reported occasional occurrence of super-numerary nuclei in some embryo-sacs of *Ulmus americana*, but recently, Fagerlind (1938), on a critical study of figures and statements published by various workers on *Ulmus* as well as his own preparations, suspects, that, in *Ulmus*, probably, a 16-nucleate embryo-sac is developed according to the *Drusa* form of the *Perperomia* type. In 1916, Dahlgren described abnormal cases of embryo-sacs in *Armeria alpina* and *A. plantaginea*. The embryo-sacs showed three synergids, one egg cell, three polar nuclei and five antipodals or four synergids, two egg cells, two polar nuclei and three antipodals. Next, Ekstrand (1918) recorded a few abnormal embryo-sacs in *Plantago major*. One of them showed seven cells in the egg apparatus, two polar nuclei and seven antipodals and another seven cells in the egg apparatus and three antipodals. In still another case, he found only three cells in the egg-apparatus and seven antipodals. Chiarugi (1925), working on *Tuberaria guttata*, found an abnormal embryo-sac with two synergids, each of them containing three big nuclei and one of them a satellite nucleus also in addition. The egg cell showed the normal form, but the secondary nucleus was divided into three nuclei. Modilewski (1925) recorded a few cases of embryo-sacs in *Allium odorum* showing three synergids, one egg cell, two polar nuclei and five or six antipodals. Maheshwari (1941) mentions a few more cases. According to him,

Gerassimova (1933), in *Crepis capillaris*, and Poddubnaja-Arnoldi and Dianowa (1934), in *Taraxacum kokosaghys*, noted occasional occurrence of 2–4 egg cells in addition to the other elements of the embryo-sac. Martinoli (1939) also occasionally found 9-nucleate and sometimes even 10-nucleate embryo-sacs in *Pyrethrum cinerariæfolium*. The author states that the extra nucleus in the 9-nucleate sac is formed due to a division of the egg and that it (extra nucleus) migrates to the centre of the embryo-sac. In the 10-nucleate sacs, he found three nuclei in the micropylar end, two in the centre of the sac (one of them being the additional cell derived from the division of the egg and the other being the nucleus formed by the fusion of the two normal polar



Figs. 30–34. *Thymelæa arvensis*.—Fig. 30. Egg fertilisation. Fig. 31. L.S. of embryo-sac in which the egg fertilisation is completed and the triple fusion is not yet completed. Fig. 32. Embryo-sac showing initial stages of endosperm formation. Fig. 33. Slightly advanced stage, the embryo-sac elongates very much, and the antipodals are left behind. Fig. 34. Nuclear endosperm. Fig. 30, ×1250; Fig. 31. ×560; Fig. 32, ×395; Fig. 33, ×265; Fig. 34, ×53.

nuclei) and five in the chalazal end. P. C. Joshi (1935) found in *Thylacospermum rupifragum* in the ovules of an abnormal ovary some abnormal embryo-sacs with 15, 16, 18 and 21 nuclei, in addition to 4- and 8-nucleate sacs. Usually in this species, an 8-nucleate embryo-sac is developed according to the normal type.

OBTURATOR

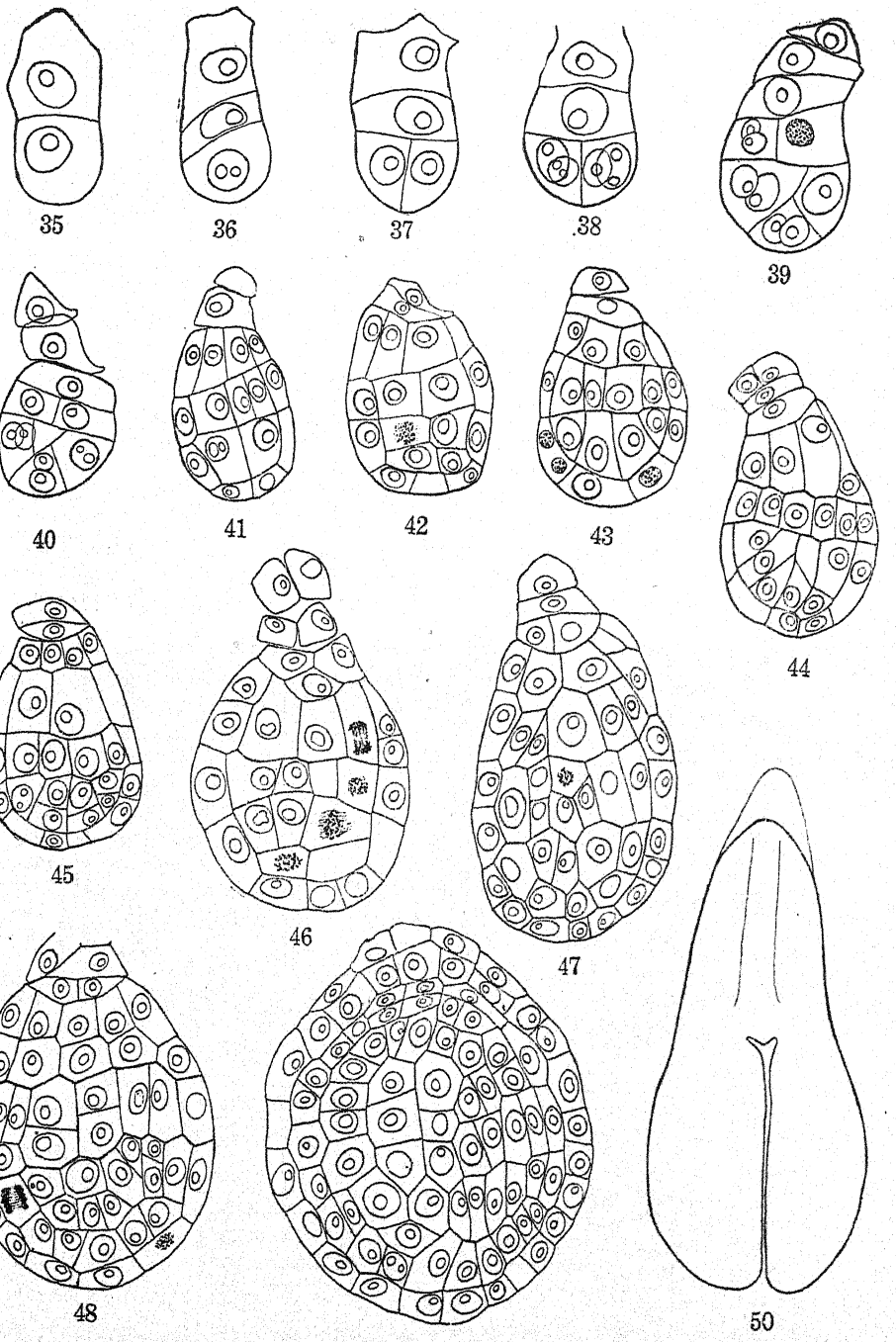
Early during the development of the ovule, the cells lining the basal part of the stylar tissue grow out into elongated cells and traverse the short space between the micropyle of the ovule and roof of the carpel (Figs. 15 and 16). All these elongated cells are rich in protoplasmic contents and form a more or less compact bundle converging towards the apex of the nucellus. The bundle descends down the funnel-shaped micropyle till it touches the nucellar apex. The presence of similarly developed obturators in other members of Thymelæaceæ has been known for a long time. They differ in their form, but not in origin. For instance, the obturator is more or less compact in *Thymelæa*, *Wikstræmia* and *Daphne*, while it is loosely arranged in *Peddia*, *Gnidia*, *Passerina*, etc. An obturator is developed also in the Elægnaceæ but it takes its origin from the funicle. An obturator is also present in Euphorbiaceæ, Rosaceæ, Umbelliferæ, etc., which are unrelated to each other. In all cases, however, it is undoubtedly concerned in directing the pollen tube to the micropyle.

FERTILISATION

The mature pollen grains, when shed, are 3-nucleate. They germinate on the stigma and the pollen tubes run down through the style and obturator and reach the nucellus of the ovule. After entering the embryo-sac, the pollen tube seems to proceed towards one of the synergids and not directly to the egg cell. Double fertilisation and triple fusion occur. One sperm enters the egg cell and is seen to be within the egg nucleus for some time before actually fusing with it (Fig. 30). The fusion between the egg nucleus and one of the sperms precedes the triple fusion (Fig. 31). The two polar nuclei lie side by side for a long time and fuse just before triple fusion.

ENDOSPERM

The endosperm is formed according to the nuclear type. After triple fusion, the endosperm primordium seems to divide near the antipodal end. The first division though not seen in my preparations, seems to be completed soon after the triple fusion and a few endosperm nuclei are always formed much before the division of the fertilised egg (Figs. 32 and 33). The nuclei migrate upwards and the cytoplasm, though scanty, accumulates in the micropylar and chalazal ends but remains thin on the sides. The chalazal accumulation is more prominent and the endosperm nuclei lie embedded in it (Fig. 34). The endosperm nuclei undergo further divisions and increase in number. The endosperm remains cœnocytic for a pretty long time, but ultimately becomes cellular (Fig. 18). The central vacuole, however is never filled up with cells.



Figs. 35–50. *Thymelæa arvensis*.—Various stages in the development of the embryo. Figs. 35–49, ×560; Fig. 50, ×53.

EMBRYO

The first division in the fertilized egg is transverse and takes place only after some endosperm nuclei have been formed. As a result of this division in the oospore, an apical cell and a basal cell are formed (Fig. 35). One more transverse division results in a 3-celled proembryo. No mitotic figure has been observed to enable me to say definitely whether it is the basal or the apical cell that undergoes the second division, but from thickness of the walls separating the three cells, it appears to have taken place in the apical cell (Fig. 36). Fuchs (1938) is also of the same opinion though no actual mitotic figure has been observed even in her preparations. Usually the proembryo remains 3-celled till the appearance of the first longitudinal wall dividing the apical cell (Fig. 37). Further increase in the length of the embryo takes place, always after the appearance of a longitudinal wall in the apical cell as a result of transverse division in either the basal or the middle cell of the proembryo. Due to lack of mitotic figures in the preparations, it is not possible to say definitely whether such further increase in the length of the embryo is due to transverse divisions in the basal or the middle cell of the proembryo.

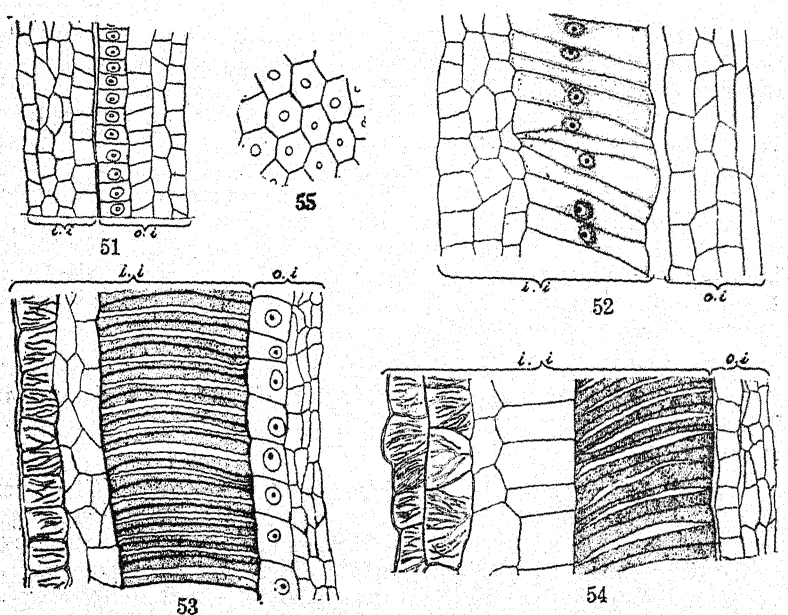
The embryo proper develops usually from four apical cells of the proembryo (Figs. 40–49). All these four cells, however, are not formed when the first longitudinal division takes place in the apical cell. The III and IV cells from the apex taking part in the formation of the embryo proper are formed at about the time when the sub-apical cell divides longitudinally or soon after it completes the division (Fig. 39). Such a belated differentiation of some cells taking part in the formation of the embryo-proper has been recorded by Kajale in *Boerhaavia diffusa* and *B. repanda* (Kajale, 1938). It is worthy of note that in the above referred species of Nyctaginaceæ also, four cells of the proembryo take part in the formation of the embryo proper (including hypophysis). The other families of Myrtales, in which detailed development of the embryo is known, differ from Thymelæaceæ in the fact that mainly the apical cell takes part in the formation of the embryo proper, the sub-apical cell only contributing to the formation of the hypophysis.

The further development of the embryo also resembles that described by Kajale (1938) in *Boerhaavia diffusa* and *B. repanda*. The four cells taking part form the different parts of the embryo as follows :—

The apical cell forms the cotyledons and the plumule. The second and third cells from the apex give rise to hypocotyl and the major part of the radicle (Figs. 46–49). The fourth cell forms the hypophysis which forms the apex of the radicle (Figs. 44, 46–49). A case however, showing that five cells may also take part in forming the embryo has been noted (Fig. 45). In such a case II, III and IV cell tiers from the apex take part in the development of the hypocotyle and radicle.

The four cells or the tiers to which they give rise do not develop simultaneously during the differentiation of the embryo. The apical-most is the first one to divide (Figs. 37, 38). Only when it forms the quadrants, the sub-apical one undergoes the first division in a longitudinal manner. It is soon followed by a longitudinal division in the third cell from the apex. By this time the fourth cell may not be even differentiated. The fourth cell is differentiated only when the dermatogen is formed in the third tier (Figs. 42, 44). Their further differentiation is given below :—

The apical cell undergoes the longitudinal division while the proembryo is only 3-celled (Fig. 37). Then one more longitudinal division in it in a plane at right angles to the first leads to the formation of the quadrants (Fig. 38). Till now, the other cells of the proembryo do not show either a transverse division or a longitudinal division. One of the quadrant cells in the apical tier undergoes an anticlinal division (Fig. 39) and the cells get arranged as if separated by oblique walls (Fig. 39). In some of the quadrants of the same tier periclinal divisions begin to take place resulting in the differentiation of the dermatogen (Fig. 40). At about this time the first longitudinal wall appears in the sub-apical cell (Fig. 40) and is soon followed by a longitudinal division in the third cell from the apex. Mostly before the first longitudinal division takes place in the third cell, the differentiation of the fourth cell taking part in the formation of the



Figs. 51–55. *Thymelæa arvensis.*—Figs. 52–54. Longitudinal sections showing various stages in the development of the seed-coat. Fig. 55. T.S. of woody palisade layer of the inner seed-coat. Figs. 51 and 52, ×373 ; Figs. 53 and 54, ×263 ; Fig. 55, ×373.

embryo proper takes place. The differentiation of dermatogen in the apical cell is soon followed by differentiation of dermatogen in the second and third tiers (Figs. 41, 43). Further multiplication of the cells inside the dermatogen takes place first of all in the apical tier in which first longitudinal or oblique walls are formed (Fig. 44). The cells so formed divide by transverse walls and result in two tiers of cells (Figs. 44–45). Similar multiplication of cells follows in the second and third tiers (Figs. 46 and 47). The cells of the second tier are shorter when compared to those composing first and third tiers (Figs. 44–46).

The differentiation of periblem and plerome follows that of dermatogen. The periblem and plerome are differentiated after a few periclinal divisions in the inner cells, while the dermatogen cells divide only in an anticlinal manner.

The cell lying next to the third cell (or tier) from the apex seems to divide transversely (Fig. 43). The daughter cell towards the apex forms the hypophysis and that towards the base adds to the length of the suspensor. The hypophysis divides anticlinally and periclinally and forms the apex of the root and the root-cap (Figs. 46–49).

The suspensor is very short and is usually made up of 2 or 3 cells (Figs. 44, 46). One, two or all of the three cells, in the later stages, may divide longitudinally and make the suspensor partly or wholly 2-seriate (Figs. 44 and 46). At about the time of the completion of the root-apex or slightly before that time, the cells of the suspensor become loose from each other (Fig. 46). Usually the suspensor cannot be seen in the advanced stages of the embryo. The embryo then grows in size. The cotyledons and stem apex are differentiated at the apex. The embryo is straight (Fig. 50).

Structure of the Seed-coat

During its development, the embryo destroys the whole of the endosperm and later on the nucellus, but one or two layers of nucellus persist. So there is present a vestige of perisperm in the seed. As already mentioned, due to their growth in the micropylar region, the integuments come together and the funnel-shaped micropyle becomes narrow.

To start with the integuments are mostly three cells in thickness. In the fertilised ovules, each of them becomes four cells in thickness (Fig. 51). The inner epidermis of the outer integument consists of prominent cells which have rich protoplasmic contents (Fig. 51). In later stages of development the cells of the outer epidermis of the inner integument become very much elongated (Fig. 52). In the seed, cells of this layer become very much thickened and their cavities are greatly reduced (Figs. 53, 54 and 55). The cell walls also become lignified and the cells form a woody palisade layer (Figs. 53 and 54). The cells composing one or two of the innermost layers of the inner seed-coat develop fibrous thickenings on their walls such as are seen in the cells of endothecium in the anther wall. The cells composing the outer

seed-coat become greatly stretched and form the thin outer seed-coat (Fig. 54).

Summary

The structure and development of the anther, pollen, ovule, embryo-sac, endosperm, embryo and seed-coat of *Thymelæa arvensis* Lamk. are described.

The anther development follows the normal course. The wall of the anther at first consists of the epidermis, endothecium, a single middle layer and the tapetum. The epidermis is greatly thinned out due to stretching in mature anthers and the single middle layer between the tapetum and the endothecium gets crushed. The primary sporogenous cells usually become the pollen mother cells. The pollen grains at the shedding stage are 3-nucleate. The sperms are elongated. Starch is present in the pollen grains. The exine shows reticulate sculpture and many germ pores. Two cases of pollen grains with more than three nuclei are described.

The solitary ovule is anatropus and two-integumented. The micropyle is formed by the inner integument. The epidermal cells of the nucellus undergo periclinal divisions and form a 2–3 cells thick cap. The archesporium in the ovule consists of a solitary sub-epidermal cell, which cuts off a primary parietal cell. The latter forms two-layers of parietal tissue which are crushed later by the developing embryo-sac. A linear tetrad of megaspores is formed. The chalazal-most megaspore gives rise to an 8-nucleate embryo-sac according to the normal type. The antipodals increase in number to about 25–30 and persist, although in a degenerate state, a long while after fertilisation. An exceptional embryo-sac with more than 8 nuclei is described.

There is a chalazal conducting strand of elongated cells in the nucellus. An obturator is developed from the base of the style. This descends down into the micropyle and helps to lead the pollen tube towards the embryo-sac.

Fertilisation is porogamous. Double fertilisation and triple fusion occur.

Endosperm is formed according to the nuclear type, but becomes cellular in the later stages. It is completely consumed by the embryo in the mature seed.

Embryo development has been followed in detail. A 3-celled proembryo is formed at first. Four cells take part in the formation of the embryo (including hypophysis), as in *Boerhaavia*. All these four cells, however, are not differentiated at the same time. Only after the apical cell of the embryo completes the first longitudinal division and forms the quadrants the third and fourth cells taking part in the formation of the embryo are differentiated. The apical cell forms the cotyledons and the plumule. The sub-apical cell and the one below it form the hypocotyl and large part of the radicle, while the fourth cell forms the apex of the root and the root-cap.

The seed-coats are two in number. The outer is 4 cells in thickness and membranous. The inner is usually 5 cells thick. Its outermost layer consists of thick-walled palisade cells. The middle cell layers are parenchymatous. The cells of the two innermost layers develop fibrous thickenings.

In conclusion, I wish to experss my sincere thanks to Prof. A. C. Joshi, D.SC., F.N.I., for his helpful suggestions and criticism during the investigation. I am also indebted to him for the material used in this investigation.

LITERATURE CITED

Beauregard, M. (1877) .. "Structure et développement du fruit des *Daphne*," *Bull. Soc. bot. France*, **24**, 385–87 (Quoted in Fuchs, 1938).

Bhargava, H. R. (1936) .. "The Life-history of *Chenopodium album* Linn.," *Proc. Ind. Acad. Sci.*, **B 4**, 3, 179–200.

Brongiart, R. (1826) .. "Zeugung des Pflanzenembryos," *Browns-Verm Bot. Schr.*, **4**, 234–317 (Quoted in Fuchs, 1938).

Capus, G. (1878) .. "Anatomie du tissue conducteur," *Ann. Sci. nat. Bot.*, Ser. **6**, 7, 209–91 (Quoted in Fuchs, 1938).

Coulter, J. Mc., and Chamberlain, C. J. (1903) *Morphology of Angiosperms*, New York.

Dahlgren, K. V. O. (1915) .. "Uber die Uberwinterungsstadien der pollen-säcke, und der samenanlagen einiger Angiospermen," *Svensk. Bot. Tidskr.*, **9**, 1–12 (Quoted in Fuchs, 1938).

Dutt, N. L., and Subba Rao, K. S. (1933) "Observations on the cytology of the sugarcane," *Ind. Jour. Agr. Sci.*, **3**, 37–56.

Fuchs, A. (1938) .. "Beitrage zur embryologie der Thymelæaceæ," *Osterr. Bot. Zeitschr.*, **87**, 1, 1–41.

Guèrin, P. (1913) .. "Lé integument séminal et lés trachees nucellaires des Thymélaeacēes," *C. R. acad. Paris*, **156**, 398–400.

——— (1915) .. "Reliquiæ Treubianæ. I. Recherches sur la structure de l'ovule et de la graine des Thymélaéacées," *Ann. Jard. bot. Buitenzorg*, **29**, 3–33.

Hofmeister, W. (1849) .. *Die Enstehung des embryos der Phanerogamen*, Leipzig (Quoted in Fuchs, 1938).

Joshi, A. C. (1937) .. "A study of pollen in the Thymelæaceæ," *Proc. Ind. Sci. Congress*, **24**, Section 4 (Botany Abstracts).

—— and Venkateswarlu, J. (1935) "Embryological studies in the Lythraceæ. I. *Lawsonia inermis* Linn.," *Proc. Ind. Acad. Sci.*, **B 2**, 5, 481–93.

——— (1935 *b*) .. "Embryological studies in the Lythraceæ. II. *Lagerstræmia* Linn.," *ibid.*, **6**, 523–34.

——— (1936) .. "Embryological studies in the Lythraceæ, III," *ibid.*, **3**, 5, 377–400.

Joshi, P. C. (1935) .. "Some abnormal ovules and embryo-sacs of *Thylacosperm rupifragum* Shrenk," *Curr. Sci.*, **3**, 11, 560–61.

——— (1936) .. "Contribution to the life-history of *Stelleria media* L.," *Proc. Ind. Acad. Sci.*, **B 3**, 8–22.

Kajale, L. B. (1938) .. "Embryo and seed-development in the Nyctaginaceæ. I. Studies in the genus *Boerhaavia*," *Jour. Ind. Bot. Soc.*, **4**, 243–54.

Kajale, L. B. (1940) .. "A contribution to the embryology of Amarantaceæ," *Proc. Nat. Inst. Sci.*, **6**, 4, 597–625.

Kausik, S. B. (1940) .. "Structure and development of the ovule and embryo-sac in *Lasiosiphon eriocephalus* Dcne," *ibid.*, **1**, 117–32.

Maheshwari, P. (1941) .. "Recent work on the types of embryo-sacs in angiosperms—A Critical Review," *Jour. Ind. Bot. Soc.*, **5 and 6**, 229–61.

Mauritzon, J. (1939) .. "Contributions to the embryology of the orders, Rosales and Myrtales," *Lunds univ. Arsskrift. N.F. And.*, **2**, 35, 120.

Osawa, I. (1913) .. "On the development of the pollen grain and embryo-sac of *Daphne* with special reference to the sterility in *Daphne odora*," *Jour. Coll. Agr. Tokyo*, **5**, 4, 237–64.

Prohaska, K. (1883) .. "Der Embryo sack and die Endosperm bildung in der gattung *Daphn.*," *Bot. Ztge.*, **41**, 865–68. (Quoted in Osawa, 1913, and Fuchs, 1938).

Schnarf, K. (1929) .. *Embryologie der Angiospermen*, Berlin.

——— (1931) .. *Vergleichende Embryologie der Angiospermen*, Berlin.

Stephens, E. L. (1909) .. "The Embryo-sac and embryo of *Geissoloma marginata*," *New Phyto.*, **8**, 345–48.

Strasburger, E. (1884) .. "Die Endospermbildung bei *Daphne*," *Ber. d. deutsch. bot. Ges.*, **2**, 112–14 (Quoted in Osawa, 1913, and Fuchs, 1938).

——— (1885) .. "Zu *Santalum* und *Daphne*," *ibid.*, **3**, 105–13 (Quoted in Osawa, 1913, and Fuchs, 1938).

——— (1909) .. "Zeitpunkt der Bestimmung des Geschlechts, Apogamie, Partheno-genesis und Reduktionsteilung," *Histol. Beitr.*, Heft 7, Jena (Quoted in Fuchs, 1938, and Schnarf, 1931).

——— (1910) .. "Chromosomenzahl," *Flora*, **100**, 330–46 (Quoted in Schnarf, 1931).

Venkateswarlu, J. (1937*a*) .. "Structure and development of the embryo-sac of *Pemphis acidula* Forst.," *Jour. Ind. Bot. Soc.*, **16**, 5, 259–62.

——— (1937 *b*) .. "A contribution to the embryology of Sonneratiaceæ," *Proc. Ind. Acad. Sci.*, **B**, 206–23.

Vesque, J. (1879) .. "Nouvelles recherches sur le développement des phanerogames angiospermes," *Ann. Sci. Nat. bot.* Ser. 6, 8, 261–390 (Quoted in Fuchs, 1938).

Winkler, H. (1904) .. "Die Parthenogenesis bei *Wikstræmia indica* (L.) C. A. Mey," *Ber. d. deustch. bot. Ges.*, **22**, 573–80 (Quoted in Schnarf, 1921).

——— (1906) .. "Botanische Untersuchungen aus Buitenzorg. II. Uber Parthenogenesis bei *Wikstroemia indica*," *Ann. Jard. Buitenzorg*, **2**, 5, 208–76 (Quoted in Fuchs, 1938, and Schnarf, 1931).

Yamaha, G. (1926) .. "Uber die zytokinese beider pollen tetradenbildung zugleich weitere Beiträge. Zur kenntnis über die zytokinese im, Pflanz enrieche," *Jap. Jour. Bot.*, **3**, 139–62 (Quoted in Schnarf, 1931, and Fuchs, 1938).

PHYSIOLOGICAL STUDIES ON SOME MEMBERS OF THE FAMILY SAPROLEGNIACEÆ

III. Nitrogen Requirements*

By K. S. Bhargava

Birla College, Pilani

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Introduction

The essentiality of a proper source of nitrogen, which is an important limiting factor in the nutrition of fungi, has been well recognised. Experimental data on their nitrogen needs have continued to accumulate during the last 15 years. Robbins (1937) has recently pointed out that fungi fall into four groups, when classified on the basis of their ability to assimilate various forms of nitrogen. According to him "Nitrogen fixing organisms" are such as are capable of assimilating nitrogen as gaseous nitrogen, nitrates, ammonium salts and organic nitrogenous compounds. Under the second group come the "Nitrate ammonium organisms" which are capable of assimilating nitrogen as nitrates, ammonium salts and organic nitrogenous compounds but are incapable of assimilating gaseous nitrogen. The third group consists of "Ammonium organisms" which can assimilate nitrogen as ammonium salts and organic nitrogen but are incapable of assimilating gaseous nitrogen and nitrates. The last category comprises the "organic nitrogen organisms" which are capable of assimilating organic nitrogenous compounds only.

The study of various genera belonging to the family Saprolegniaceæ seems to have been much neglected as regards this important factor. Volkonsky (1933, 1934) studied the suitability of some nitrogenous substances on a few of them but he too did not give a relative value of various nitrogenous substances used by him. A little later Leonian and Lilly (1938) found that *Saprolegnia parasitica* along with some others was unable to grow with ammonium nitrate and required an amino acid for its growth.

The present investigation was undertaken with the aim of clarifying and adding to our knowledge the nitrogen needs of some members of the family Saprolegniaceæ, *viz.*, *Achlya* sp., *Brevilegnia gracilis* v. Eek., *Isoachlya anisospora* var. *indica* Sak. et Bhar., *Saprolegnia delica* Coker and *S. monoica* Pringsh. hitherto uninvestigated.

*Part of Thesis approved for the degree of Doctor of Philosophy at the University of Allahabad, in 1943.

METHODS

The methods and technique employed in this investigation were the same as described in an earlier paper (Bhargava, 1945). The basal medium consisted of 0·5 gm. each of KH_2PO_4, $MgCl_2 \cdot 6\,H_2O$, 0·17 gm. of Na_2S, 5 gm. of dextrose and 1,000 c.c. of double distilled water. The source of sulphur in the case of *Brevilegnia gracilis* was in the form of K_2SO_4 (0·5 gm. per lit.) instead of Na_2S. Magnesium chloride was prepared by the action of pure hydrochloric acid on magnesium ribbon, the commercial reagent grade chemical (Proanalysis of Merck) being of no value in these experiments, as it contained traces of ammonia.

Because of the prohibitive prices of various amino acids only qualitative experiments were carried out in the first instance. The cultures were grown in culture tubes containing 10 c.c. of the nutrient medium. To compare the relative value of nitrogenous compounds as source of nitrogen only a few representatives were taken for quantitative experiments.

Various nitrogenous compounds (inorganic and organic) were added singly to the basal medium in amounts calculated to furnish 700 mg. of nitrogen per litre. Trihydroxy triethylamine, tyrosin and cystin were tried in 0·1%, 0·1% and 0·05% concentrations respectively because of their low solubility. Peptone was used in 0·1% concentration because of its unknown constitution.

EXPERIMENTAL

To the basal medium were added the following compounds singly before autoclaving :—

Inorganic nitrogen.—

Ammonium nitrate, ammonium chloride, ammonium sulphate, sodium nitrate and sodium nitrite.

Organic nitrogen.—

Amino acids :

(*a*) Mono-amino derivatives of aliphatic mono-carboxylic acids—
Glycin, *d*-alanin, *d*-valin and *l*-leucin.

(*b*) Diamino derivatives of aliphatic mono-carboxylic acids—
d-arginin and *d*-lysin.

(*c*) Mono-amino derivatives of aliphatic dicarboxylic acids—
l-asparatic acid, *d*-glutamic acid and asparagin.

(*d*) Aromatic amino acids—
l-phenyl-alanin and tyrosin.

(*e*) Heterocyclic amino acids—
Histidin, tryptophane and prolin.

(*f*) Thioamino acids—
Cystin and cystein hydrochloride.

Amides : Acetamide.

Amines : Urea and trihydroxytriethylamine.

Proteins : Peptone.

The various media thus obtained were inoculated with the fungi. The basal medium alone served as control. It was found that all the nitrogenous substances, except sodium nitrate, sodium nitrite, glycin, arginin, urea and trihydroxytriethylamine were able to supply nitrogen necessary for the growth of *Achlya* sp., *Isoachlya anisospora* var. *indica* and *Saprolegnia monoica*. In the case of *Brevilegnia gracilis* sodium nitrate, glycin and trihydroxyethyl-amine also supported growth, while acetamide was valueless.

To compare the relative value of some of the easily available nitrogenous compounds, the media were poured in flasks and inoculated with the fungi. Table I gives a résumé of this experiment.

TABLE I

Dry weight (in mg.) of fungal colonies grown on 25 c.c. of the basal medium containing organic and inorganic nitrogenous compounds

Period of incubation = 21 days. Temperature = 25° C.

Compounds	*Achlya* sp.	*B. gracilis*	*I. anisospora* var. *indica*	*S. delica*	*S. monoica*
Ammonium nitrate ..	12·0	20·0	10·0	5·0	25·0
Amm. chloride ..	10·2	10·6	9·5	3·5	21·6
Amm. sulphate ..	9·8	10·0	8·6	4·5	15·0
Sodium nitrate ..	..	18·3	..	..	..
Acetamide ..	5·0	..	4·3	5·0	3·0
Glycin ..	..	27·3	..	..	..
Alanin ..	12·3	32·6	21·6	3·5	6·5
Glutamic acid ..	15·0	23·0	37·3	15·0	29·0
Asparagin ..	5·8	28·3	13·3	15·0	10·0
Basal medium (control) ..	..	..	..	..	..

The results summarised in Table I show that of all the nitrogenous substances tested, glutamic acid is generally the best and acetamide the poorest source of nitrogen.

Effect of sodium acetate on the utilisation of glycin.—Enhancement of growth of *Leptomitus lacteus*, a watermold, by the addition of glycin in a medium suggested to Schade (1940) that it might prove an available nitrogen source if any suitable carbon could be supplied. He found that an acetate, which is an oxidisable substrate, provides a very favourable source for glycin utilisation in the case of *L. lacteus*. To see if acetate would induce growth of the organisms in this case too (where a good source was already present), the following media were prepared :

1. KH_2PO_4 .. .. 0·5 gm.
   $MgCl_2 \cdot 6\,H_2O$ .. .. .. 0·5 „
   Na_2S .. .. 0·15 „
   Glycin .. .. 3·0 „
   Distilled water .. .. .. 1,000 c.c.
2. Medium 1+glucose .. .. 5·0 gm.
3. Medium 2+sodium acetate .. 2·0 „
4. Medium 1+sodium acetate .. 2·0 „

These were inoculated with *Achlya* sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia monoica* and *S. delica*. On examining the cultures after seven days of inoculation, it was found that there was no growth.

Effect of molybdenum on the utilisation of nitrates.—Steinberg (1937) in his extensive and well-balanced studies stressed the importance of trace elements in the nutrition of fungi. He found that molybdenum was required in a greater degree by the organisms when a nitrate and not ammonia or organic nitrogen was the source. The following media were prepared to see the possible effect, if any, of molybdenum on the utilisation of nitrate :

1.	KH_2PO_4	0·5 gm.
	$MgCl_2 \cdot 6\ H_2O$	0·5 „
	Na_2S	0·15 „
	Dextrose	5·0 „
	$NaNO_3$	4·2 „
	Distilled water	1,000 c.c.

Achlya sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia delica* and *S. monoica* which showed no growth on a medium containing $NaNO_3$ were grown on media 1 and 2. It was found that there was no growth in any case.

Discussion

Since the fungi used in the present study are unable to grow on a synthetic medium lacking in the source of nitrogen, it is evident that nitrogen is essential for the growth of these organisms. As to the form of nitrogen suitable for their growth, *Achlya* sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia delica* and *S. monoica* are unable to utilise nitrite or nitrate, but show good response to ammonium as well as organic nitrogen. Therefore, they very well fit in the "Ammonium organisms" placed separately by Robbins (1937). Steinberg (1937) has shown that molybdenum is essential for the activation of nitrate reductase in the reduction processes whereby nitrates are reduced to ammonia. That the non-utilisation of nitrates is not due to the absence of molybdenum is shown clearly by the absence of growth of these organisms on the medium to which molybdenum had been added in the form of sodium molybdate. *Brevilegnia gracilis* behaves differently and is able to utilise nitrates in addition to ammonium and organic nitrogen. It comes under "Nitrate ammonium organisms". This difference may easily be explained on the basis of the different habitat of the fungi ; *B. gracilis* is a parasite on the roots while the other forms are watermolds.

The general opinion about the availability of nitrite nitrogen is that it is toxic for the growth even in dilute solutions, and that if the fungi use it, they do so with difficulty (Ohtsuki, 1936 ; Sakaguchi and Wang, 1936 ; Wang, 1937 and others). Of the 25 fungi used by them, Leonian and Lilly (1938) obtained fair amount of growth of only one organism, *viz.*, *Blakeslee trispora* with sodium nitrate as source of nitrogen.

Of the organic nitrogen, proteins which are the result of a combination of different amino acids are necessary for the building up of the body of an organism. This holds true for fungi as well. Since the organisms studied above are able to utilise nitrogen from NH_4NO_3 or a single amino acid as the only source of nitrogen in a nutrient medium, it can safely be concluded that they manufacture their own amino acids from these substances. Leonian and Lilly (1938) have reported that *Blakeslee trispora, Phycomyces nitens, Pythium oligandrum* and *P. polymastum*, etc., require only one favourable amino acid as the source of nitrogen for good growth. These organisms resemble *Pythium arrhenomanes, P. deliense, P. graminicolum* and *P. mamillatum* (Saksena, 1940) which are able to grow with NH_4NO_3 or one amino acid.

Amines are generally poor source of nitrogen. Schade (1940) also found them valueless for *Leptomitus lacteus*. Volkonsky (1933, 1934) reported that acetamide was not assimilated by *Saprolegnia dioica* while glycocoll and urea were utilised by this fungus.

The best growth on glutamic acid can be attributed, as already explained by Waksman and Lomanitz (1925), to the much larger ratio of the carbon to the nitrogen and that glutamic acid is very favourable to respiration, resulting in the formation of very little volatile acids. This is further explained by the fact that when the ratio between carbon and nitrogen is high, the amount of ammonia produced will be less because the fungus will continue to grow and derive its energy from the available carbohydrate, and ammonia which is a waste product in carbon metabolism will be utilised resulting in greater amount of growth.

That the failure of *Achlya* sp., *Isoachlya anisospora* var. *indica Saprolegnia delica* and *S. monoica* to grow on glycin is not due to the absence of proper carbohydrate or any oxidisable substance is clear from the experiment where these fungi failed to grow on a medium to which acetate had been added in the presence of glycin.

Summary

Achlya sp., *Isoachlya anisospora* var. *indica, Saprolegnia delica* and *S. monoica* are unable to utilise nitrite or nitrates as source of nitrogen but thrive on ammonium and organic nitrogenous compounds. Glutamic acid serves as the best source, while acetamide is the poorest. *Brevilegnia gracilis* is able to obtain nitrogen from nitrates as well. Addition of molybdenum does not help in the assimilation of nitrates. Glycin, which is a good source of nitrogen for *B. gracilis*, is valueless for the growth of others, and remains so even in the presence of an oxidisable substrate.

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LITERATURE CITED

Bhargava, K. S. (1945) .. "Physiological studies on some members of the family Saprolegniaceæ. II. Sulphur and Phosphorus requirements", *Proc. Ind. Acad. Sci.*, 1945 (in press).

Leonian, L. H., and Lilly, V. G. (1938) "Studies on the nutrition of fungi. I. Thiamin, its constituents, and the source of nitrogen," *Phytopath.*, **28**, 531–38.

Ohtsuki, T. (1936) .. "Untersuchungen über die Nitritverwendung und die Nitrat reduktion bei Schimmelpilzen," *Japan Jour. Bot. (Tokyo)*, **8**, 269–93.

Robbins, W. J. (1937) .. "Assimilation by plants of various forms of nitrogen," *Amer. Jour. Bot.*, **24**, 243–50.

Sakaguchi, K., and Wang, Y. (1936) "Studies on the assimilation of nitrites by fungi," *Bull. Agr. Chem. Soc. Japan*, **12**, 63–69.

Saksena, R. K. (1940) .. "The nutrition of some species of the genus *Pythium* on synthetic liquid media," *Proc. Nat. Acad. Sci., India*, **10**, 1–13.

Schade, A. L. (1940) .. "The nutrition of *Leptomitus lacteus*," *Amer. Jour. Bot.*, **27**, 376–84.

Steinberg, R. (1937) .. "Rôle of molybdenum in the utilisation of ammonia and nitrate nitrogen by *Aspergillus niger*," *Jour. Agr. Res.*, **55**, 891–902.

Volkonsky, M. (1933) .. "Sur les conditions de culture et le pouvoir de synthese de *Saprolegnia* sp. Etudé qualitative de l'alimentation carbonéé, azotéé et sulphurèa," *Ann. Inst. Pasteur*, **50**, 703–30.

——— (1934) .. "Sur les nutrition de quelques champignons saprophytes et parasites," *ibid.*, **52**, 76–101.

Waksman, S. A., and Lomanitz, S. (1925) "Contribution to the chemistry of decomposition of proteins and aminoacids by various groups of micro-organisms," *Jour. Agr. Res.*, **30**, 263–81.

Wang, Y. (1937) .. "Assimilation of nitrites by fungi. III," *Jour. Agr. Chem. Soc. Japan*, **12**, 877–84 (Original not seen).

STUDIES IN THE BIOLOGY OF WOOD-ROTTING FUNGI OF BENGAL

BY SACHINDRANATH BANERJEE AND BIMAL KUMAR BAKSHI

Department of Botany, Calcutta University

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I. INTRODUCTION

THE rôle of fungi as agents for the destruction of timber was established by Robert Hartig.[20, 21] Wood-rotting fungi are very common in the forests of India, Europe, America and elsewhere. In many cases, a large percentage of timber is found infected. This has led to an increasingly larger number of workers devoting themselves to the study of tree and timber diseases. Among these may be mentioned Ward,[39] von Schrenk,[37, 38] Lyman,[25] White,[40] Rhoads,[31] Long and Harsch,[24] Buller,[15, 16] Fritz,[19] Baxter[4] and Mounce.[26]

The study of cultural characters for distinguishing wood-rotting fungi is also of great importance. Until recently, identification of the causative organism producing the rot was made from the sporophore found on the external surface. They are, however, not always present on rotten wood, and even when present, may not belong to the fungus actually destroying the internal tissues. In such cases, a pure culture of the fungus from the infected wood can be made.

The most important contribution to the study of this subject in India comes from Bose.[6–13] He has given a systematic account of the Bengal Polyporaceæ and described the life-histories and cultural characters of some of them. But a vast number of wood-rotting fungi still await investigation. The present paper makes a contribution to our knowledge of the life-histories and cultural characters of six wood-rotting polypores of Bengal, namely, *Polyporus brumalis* (Pers.) Fr., *P. friabilis* Bose, *P. rubidus* Berk., *P. ochroleucus* Berk., *Polystictus steinheilianus* Berk. and Lév., and *Merulius similis* B. et Br. Geographical distribution and occurrence of these species have been compiled from the reports of Berkeley,[5] Bresadola[14], Butler and Bisby,[17] Lloyd,[22,23] undkur,[27] Oudemans,[28] Petch,[29] Rabenhorst,[30] Saccardo,[33] Theissen[36] and Bose.[6–10]

II. CULTURAL METHODS AND CONDITIONS OF GROWTH

A. *Types of culture*

The initial cultures of all the species under investigation were made either from spores or from the tissue of the sporophore. A spore-culture was, however, preferred to a tissue-culture because in the former case the life-history of the fungus from spore to spore could be studied.

The spore-discharge was obtained from a fresh sporophore on 3% sterile agar contained in the lower lid of a sterile petri-dish, while a trimmed rectangular block of the sporophore was fixed eccentrically to the inner side of its upper lid with the hymenial surface directed downwards and the whole was placed inside a moist chamber for a few hours. The spores, thus deposited, were then transferred aseptically to culture tubes containing *potato-dextrose agar* and within a day or two, several polysporous mycelia were obtained. In *Merulius similis*, polysporous mycelia could not be obtained by this method and monosporous cultures, both by the 'dilution method' and by the 'streak method' were made.

In making tissue-cultures, the technique adopted by Duggar[18] was mainly followed. Fresh, dried sporophores, however, were used, since it was noticed that contaminations, especially bacterial, were large when fresh but water-soaked fruit-bodies were taken. Tissue-cultures were also successfully made from comparatively dry older specimens, but in such cases, a large inoculum was preferred to a small one owing to the likelihood of the former containing a larger percentage of viable mycelia.

B. *Media used*

For the comparative study of cultural characters of these fungi, the following media were used: (1) *potato-dextrose agar* prepared by Fritz's method,[19] (2) *malt-extract agar* (25 gm. agar and 30 gm. malt extract in 1,000 c.c. distilled water), (3) *oat-meal-agar* (25 gm. agar and 50 gm. quaker oats in 1,000 c.c. distilled water), and (4) *Brown's potato-starch agar* (0·2 gm. aspargin, 2·0 gm. glucose, 0·75 gm. magnesium sulphate, 10·0 gm. potato-starch, 1·25 gm. potassium phosphate and 25 gm. agar in 1,000 c.c. distilled water). Eight c.c. of the medium were poured in each culture tube which was then plugged, sterilised and slanted to the same degree in order to provide surface of uniform area. The pH values of the media after sterilisation, were determined by *k*-potentiometric method and found to be 5·2, 5·2, 4·5 and 5·2 respectively.

A new medium for the cultivation of wood-rotting fungi was described by Badcock.[1] According to the author, many wood-rotting fungi which fail to fructify in other commonly used media readily produce fruit-bodies in this medium. This medium was tried successfully but 'pine wood ash' was used instead of 'Scots fir ash' which was not available.

Wood-block cultures were also made both in Roux-tubes and in Erlenmeyer flasks. Normal and healthy pieces of wood of *Mangifera indica*, *Bambusa arundinacea*, *Cocos nucifera*, *Shorea robusta*, *Ficus religiosa* and *Ceriops Roxburghiana* were cut into convenient sizes ($3'' \times \frac{3}{4}'' \times \frac{3}{4}''$), and cultures were made by Bose's method.[12] Though the fungi under consideration were mainly collected from bamboo stumps, blocks of the wood of other monocotyledonous and dicotyledonous plants were used in order to find out whether the fungi could attack these hosts as well.

C. *Light, Temperature and Humidity*

(1) *Light.*—All the cultures were exposed either to the diffused light of the laboratory or to complete darkness. In the former case, cultures were placed in an inclined position on the shelves of an almirah situated at a distance of about 15 feet away from the window and these received diffused light from morning till evening. For complete darkness, the doors of an almirah were covered with thick black papers in such a way that no light entered into it when the doors were closed.

(2) *Temperature.*—The cultures were subjected to three ranges of temperature, namely, constant temperatures of 22° C. and 33° C. and a variable temperature of 23°–27° C. of the laboratory during the months of November and December, 1941. All wood-block cultures were, however, kept only in diffused light and in ordinary room temperatures of the laboratory.

(3) *Humidity.*—The relative humidity at the above temperature conditions, was determined as 69–75% at 22° C., 35–42% at 33° C., and 35–58% at 23°–27° C.

D. *Number of tubes inoculated*

Culture tubes $6'' \times \frac{1}{2}''$ in size made of neutral glass were used for the study. In order to make the comparative study thorough and at the same time avoiding any risk, three tubes were used for each treatment and the results and conclusions were based on the average of these.

E. *Important diagnostic characters in culture*

The following characters were studied in the cultures of the various species : (1) macroscopic characters including rate of growth, texture and colour of the aerial mycelium, staining and decolouration of the medium, appearance and drying up of glistening drops of liquid, etc.; (2) microscopic characters such as types of hyphæ, their septation, branching, colour, clamp-connection, spore-formation and crystalline material. Descriptions of basidia, basidiospores or other anatomical characters in the fruit-bodies in culture have also been given. All observations were made from fresh mounts in water. Preparations of lactophenol, 50% glycerine, staining with lactophenol cotton blue were also made.

III. Descriptions of Species Investigated

1. Polyporus brumalis (*Pers.*) *Fr.*

Geographical distribution

The species is widely distributed over the tropical and temperate regions of the Old World and only in the temperate regions of the New. In North America, it is common in Canada (Manitoba), Lake Superior (Isle Royale), New Hampshire, Michigan, South-West Virginia, Ohio, Iowa, New York, Wisconsin and in the Middle and Upper Carolina. In Europe, it occurs in Great Britain, Spain, France,

Netherlands, Denmark, Czechoslovakia and Esthonia. The fungus is also found in the mountainous forests of Basehberg near Somerset East and Cape of Good Hope in South Africa ; in India, it is found in Bombay, Punjab, Bengal and Orissa. It is also found in Central Asia and Siberia in the North. In Australia, it occurs in New South Wales, Victoria and Queensland.

Occurrence

It has been recorded on several hosts, viz., *Corylus betulus* L., *C. Avellana* L., *Alnus glutinosa* Gaertn., *Betula verrucosa* Ehrh., *Fagus sylvatica* L., *Acer platanoides* L., *Aesculus hippocastanum* L., *Fraxinus excelsior* L., *Tilia cordata* Mill., and *Saccharum munja* Roxb. It has been collected by the authors from suburbs of Calcutta (Behala) where it was growing saprophytically on a dead and fallen branch of a tree.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, young cultures developed a felty mat in most cases and a sub-felty to felty mat in others. The advancing hyphæ formed a colourless appressed subfelty to felty zone. Irregular condensation of the mycelium in young cultures in darkness at 33° C. made the surface uneven. At 22° C. the cultures exhibited faint zonation. Colourless glistening drops of liquid appeared invariably in cultures 7-days-old. Later on, the mat became compact and uniform. Vigorous growth in darkness at 33° C. was manifested by the evident rolling of the mycelium on the bare glass surface. In most cultures, prominent ridges appeared on the mat making the surface uneven. After 10 days of inoculation, the mat in room temperature became granular at places, though granules in darkness were less than those under diffused light. Later on, however, these granules disappeared. Within a fortnight, the glistening drops of liquid disappeared, growth declined and the mat became thin and appressed. In some cases the mat was covered by a fresh growth of thin, colourless mycelium in old culutres. On *oat-meal-agar*, the mat under all conditions presented a subfelty to felty appearance except at 22° C., where the mat was appressed showing zonation. The advancing zones were distinctly appressed and sodden. The other characteristics as well as the later development of the mat were like that described for the previous medium. On *malt-extract-agar*, the texture was more or less similar to that on oat-meal-agar. In darkness at 33° C. and in diffused light at 22° C. the young hyphæ at the advancing zone were parallel and combed like silk. On *potato-starch-agar*, the growth was poorest in comparison to other media. The mats in most cases were thin, appressed and sodden. This texture also persisted in old cultures.

(ii) *Colour**.—On *potato-dextrose-agar*, colouration appeared after a week's growth, these being shades of cinnamon buff, light vinaceous fawn, pale ochraceous salmon, light ochraceous salmon, sorghum

* According to Ridgeway.[32]

brown and light vinaceous cinnamon. In early stages the colour confined mainly to the inoculum, the hyphæ at the advancing zone remaining colourless. Pigmentation was accentuated in diffused light and tinting in darkness appeared later. After 10-days-growth all the earlier shades disappeared and deeper tint of seashell pink, zinc orange, ochraceous orange, hays russet, mars brown, pale vinaceous drab, light buff, warm buff and orange pink appeared. Colour was best developed in room temperature (23°–27° C.) and for 33° C., pigmentation was denser in diffused light than in darkness. In 22° C. varied colouration was much pronounced in dark than in diffused light, in the latter case, pigmentation developing after a fortnight. Thus, in cold room diffused light definitely retards pigmentation, while darkness accentuates it, but in the other two temperature conditions, reverse was the case. After 21-days-growth, most of the earlier shades persisted, but a deeper shade of liver brown and orange rufus appeared. After a month's time, the shades deepened still further to deep chrome, hæmatite red and vandyke brown. On *oat-meal-agar*, the sequence of colour development as well as the range of colour were like those described above. On *malt-extract-agar*, the intensity and the range of colour were much less. The difference as regards the conditions of light and darkness, if at all present, was very slight. On *potato-starch-agar*, colouration was the poorest and developed after 21-days-growth. The tinting was very light with shades of light buff, pale ochraceous buff, sea-shell pink, pale vinaceous drab, and liver brown. The colour of the medium was changed.

(iii) *Rate of growth.*—The slants were covered with moderate rapidity in all the media, but advance in *potato-starch-agar* was slower in comparison to other media. The rate of growth was highest at 33° C., moderate at room-temperature (23°–27° C.) and comparatively less in 22° C. In general, growth in diffused light was more rapid than growth in darkness.

(iv) *Sporophore production.*—Stalks of fruit-bodies appeared in several cultures under different conditions but the first tube to fructify was on *oat-meal-agar* in diffused light at 33° C. Three stalks arose from the base, two of which were suppressed after some time while the third one developed. Later on, similar stalks appeared in other conditions but comparatively larger number fructified at incubator-temperature. The stalks always had a tendency to branch. In no case, however, pileus was formed. Bits of mycelium from 7-days-old cultures on *potato-dextrose-agar* were transferred to wood blocks in Roux-tubes as well as in flasks. Growth started within a few days with its characteristic colour and zonations as noted on agar media. The mat was thin and appressed. No fruit-bodies, however, developed on wood. Cultures were later grown on the medium advocated by Badcock[1] in Erlenmeyer flasks of 1,000 c.c. capacity each. Normal fruit-bodies with true pilei developed in about a month. A pileus, on sectioning, revealed basidia ($10–12\,\mu \times 5–6\,\mu$), each with four sterigmata bearing basidiospores ($4\,\mu \times 4\,\mu$).

(v) *Mycelium.*—Hyphæ in young and old cultures are of three types, (*a*) thin-walled, hyaline, with dense protoplasmic contents, septate, profusely branched, clamp-connections numerous, 3–4 μ broad ; (*b*) thin-walled, hyaline, with granular protoplasm, septate, sparingly branched, clamp-connections, about 2 μ broad : and (*c*) thick-walled, hyaline, unbranched and 7–8 μ broad. Chlamydospores abundant both in young and old cultures, terminal and intercalary, with granular contents, 10–12 $\mu \times 10\,\mu$. Abundant crystals of calcium oxalate are present (Text-figs. 1–5).

2. Polyporus friabilis *Bose*

Geographical distribution

Polyporus friabilis is confined to India and is very common in Bengal. Outside Bengal, it occurs in Madras and Orissa.

Occurrence

The fungus usually grows on humus being associated with rotten leaves and grasses. It has been reported to be growing on *Excœcaria agallocha* L. from Madras. The writers collected the material from dead bamboo clumps and palm stems in Calcutta, Howrah and suburbs.

Fungus in culture

(i) *Habit of growth.*—The mycelium spread rather slowly over the agar surface. On the *potato-dextrose-agar*, inoculum, in most cases, was downy or downy to velvety at the beginning and the advancing zone was evident. In 10 days, the mat became compact and felty. Best growth was obtained at 33° C., moderate in room temperature (23°–27° C.) and comparatively poor at 22° C. In the last case, growth appeared as a more or less circular patch with distinct, appressed and sodden advancing zone. In old cultures, growth was vigorous under all conditions. Rolling of the mycelium over the bare glass surface was vigorous at 33° C., less so in room temperature, while no rolling could be noticed in culutres kept at 22° C. After 35 days of inoculation the superficial mat, in all cases, presented a thick matted felt. Colourless glistening drops of liquid appeared in cultures about a month old in diffused light at 33° C. These drops assumed a beautiful pink colouration in about 2-months-old cultures. Later on, the drops dried up. On *oat-meal-agar* the habit of growth was in the main described as above. The advancing zone at room temperature was appressed and like combed hairs. At 33° C., the upper advancing zone was like a pile of velvet. On *malt-extract-agar*, the growth was comparatively poor. In room temperature, the mat was more compact in diffused light than in darkness. The downy texture gave place to cottony mat, and finally to a smooth matted felt. On *potato-starch-agar*, growth appeared to be poorest, while other characteristics were as described for *malt-extract-agar*.

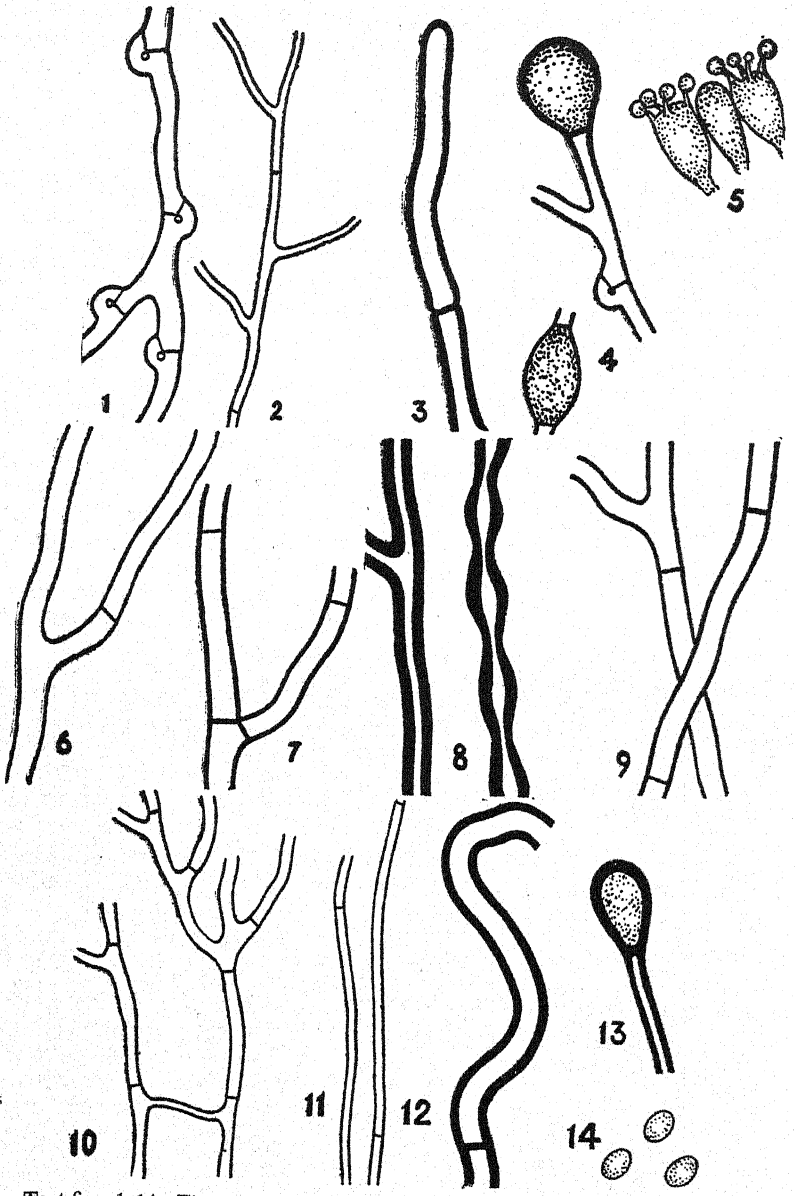
(ii) *Colour.*—On *potato-dextrose-agar*, no colour appeared in 5-days-old cultures except under diffused light at 33° C., where a patch

of deep safrano pink appeared round about the inoculum. In 10 days, colouration appeared in room temperature and at 33° C. but no tinting was noticed at 22° C. This signifies that for this fungus, a low temperature prevents pigmentation, while a higher temperature accentuates it. Again, since colour appeared earlier in diffused light and later in darkness under any condition of temperature, it suggests that light accentuates pigmentation while darkness retards it. Tinting in young cultures was mainly over the inoculum and consisted of shades of light buff, pale flesh colour to flesh colour and pale salmon colour. Other portions of the mat remained colourless, which, later on, developed pigmentation. The earlier shades, later on, deepened to sea-shell pink, salmon buff, pale ochraceous salmon, pale buff, light buff, light ochraceous buff and pale flesh colour. These shades, however, were mixed with white. In 35 days, most of the shades disappeared and deeper tints of salmon orange, light russet, vinaceous, brownish vinaceous, sweet pink and flesh colour appeared. Colour of the medium was considerably changed in old cultures. On *oat-meal-agar* colouration developed late and no colouration was noticed till the 25th day after inoculation except in diffused light at 33° C. where safrano pink, developed on the 5th day, deepened to light buff on the 10th day. The later shades approached nearly those described for the previous medium. On *malt-extract-agar*, tinting of the mycelium was poor as compared to the previous media, while on *potato-starch-agar*, colour developed only in darkness at room temperature and on diffused light at 33° C.

(iii) *Rate of growth.*—The rate of growth was best on *oat-meal-agar* and on *potato-dextrose-agar*, moderate on *malt-extraxt-agar* and least on *pottato-starch-agar*. The advance was rapid in cultures kept at 33° C., moderately high in room temperature, while at 22° C. the advance was very slow. The dual effect of *potato-starch-agar* medium and low temperature had a marked effect on growth, for no sign of growth could be seen in 25-days-old cultures when growth could be evident only in darkness. Growth in darkness was more than growth under diffused light under all conditions.

(iv) *Sporophore production.*—Bits of cultures from 7-days-old cultures on *oat-meal-agar* were transferred to wood-blocks in Roux-tubes and in flasks. Growth started within a fortnight. In Roux-tubes, growth was very vigorous, giving a thick woolly appearance, some mycelium at the lower part penetrated into water and formed a floating mycelial mat. In flasks, the same woolly appearance was seen. The colouration of the mycelia was typically as seen on agar media. Fruit-bodies were developed neither on agar media nor on wood-blocks.

(v) *Mycelium.*—The hyphæ from culture consists mainly of two types, *viz.*, (*a*) thin-walled, branched, septate, 4–4·7 μ broad and (*b*) thick-walled, branched, septate, somewhat beaded in appearance and 4·7–6 μ broad, Crystals of various shapes are present (Text-figs. 6–7).



Text-figs. 1–14—Figs. 1–5. *Polyporus brumalis* : 1. thin-walled hypha with clamp-connections; 2. thin-walled hypha without clamp-connections; 3. thick-walled hypha ; 4. chlamydospores ; 5. basidia with basidiospores. Figs. 6–7. *P. friabilis* : 6. thin-walled hypha ; 7. thick-walled hypha. Figs. 8–9. *P. rubidus* : 8. thick-walled hypha ; 9. thin-walled hyphæ. Figs. 10–14. *P. ochroleucus* : 10. thin-walled, much branched hyphæ ; 11. thin-walled, unbranched hyphæ ; 12. thick-walled, unbranched hypha ; 13. terminal chlamydospore ; 14. basidiospores. (×520).

3. Polyporus rubidus (*Pers.*) *Fr.*

Geographical distribution

The fungus has been collected from Brazil and Rio Grande do Sul in S. America. In India, it occurs in Calcutta and suburbs, Sinchul hills (Bengal) and Lokra hills (Assam). It is also found in Burma, Malaya Peninsula, Java, Lang, Alor and Philippine Islands.

Occurrence

It has been reported to grow on dead wood of *Alnus nepalensis* D. Don from Darjeeling. The authors have collected the fungus from dead bamboo clumps in Sonarpur, 24-Parganas, Bengal.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, and on *oat-meal-agar*, the initial growth of the superficial mycelium produced a subfelty to felty mat with a narrow and appressed advancing zone. Rolling of the mycelium on the bare glass surface in the form of long, weak hyphæ giving a silky appearance began very early in young cultures and is characteristic for the fungus. This rolling was, however, very vigorous at 33° C. Within 10 days after inoculation in room temperature (23°–27° C.) and at 33° C., the mat became compact and felty throughout due to early condensation of the mat with a pile of velvet at the upper advancing zone. At 22° C., there was less condensation of the superficial mat so that it remained loosely felty. The upper advancing zone, moreover, did not exhibit a pile of velvet as stated above. In 35-days-old cultures, the growth characters remained constant under all conditions, each tube exhibiting a semilunar raised velvety region at the upper advancing zone. No glistening drops of liquid were observed during the course of study. As the cultures dried up, the long rolling hyphæ began to recede from the base of the glass tube in the form of dry membranous sheet. On *malt-extract-agar*, the texture of the mat was floccose or floccose subfelty to felty texture in about 10 days after inoculation. At 22° C., condensation was less, so that the mat remained floccose. In 25-days-old cultures, the mat became felty in all cases. The other characteristics, namely, the formation of the pile of velvet at the upper advancing zone, rolling, &c., were like those described above.

(ii) *Colour.*—No colour developed under any condition in 5-days-old cultures. On *potato-dextrose-agar*, colour developed on the 10th day after inoculation, the shades being mainly capucine buff, and pale ochraceous buff towards the inoculum and light buff at the upper advancing zone. No colour developed in darkness at 22° C. Later on, the shades deepened to cinnamon buff, sayal brown, and ochraceous tawny. The colour of the medium was changed to mummy brown. The glass surface was stained due to tinting of the rolling mycelium over the bare glass surface. The tinting on the glass consisted mainly of shades of amber brown, yellow ochre, old gold, pinkish buff. Tinting on the pile of velvet at the upper advancing zone consisted of pale ochraceous buff to light ochraceous buff. On *oat-meal-agar* and on

malt-extract-agar, the intensity of colour was much less than those described above. On *potato-starch-agar*, colour production seemed to be least pronounced. A tinge of sayal brown developed at 33° C. in 25 days. Later on, a shade of tawny developed at 22° C. under diffused light.

(iii) *Rate of growth.*—The rate of growth of the superficial mycelium was best at 33° C., moderate at room temperature and comparatively less at 22° C. The slants were covered within a week in room temperature and at 33° C. and within 10 days at 22° C. Growth in darkness, in general, was more vigorous than that under diffused light.

(iv) *Sporophore production.*—Several wood-blocks were inoculated with the fungus both in Roux-tubes and in flasks as usual. Growth started after about a fortnight and attacked the wood vigorously. After about a month, the mycelium condensed at places forming a buff coloured area. No fructification appeared on agar media or wood-blocks.

(v) *Mycelium.*—Two main types of hyphæ can be seen in cultures, *viz.*, (*a*) thin-walled, much branched, contents hyaline, 2·5–3 μ broad and (*b*) thick-walled, distantly branched, contents hyaline, contents granular and 3–3·7 μ broad (Text-figs. 8–9).

4. Polyporus ochroleucus *Berk.*

Geographical distribution

The fungus is widely distributed, being found in the Bahamas and Brazil in S. America ; Portugal in Europe ; Portuguese Guinea and Eastern Cape Forest conservancy in Africa. In India, it occurs in Bengal, Assam (Lokra hills) and after passing through Malaya Peninsula and Molucca Islands, it extends upto Queensland, New South Wales and Tasmania in Australia. It is also found in Ceylon and Japan.

Occurrence

It has been reported to grow on dead logs at the base of the trunk of *Casuarina equisetifolia* L. and on dead trunks of *Lagerstræmia flos-reginæ* Retz.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, the young cultures developed a downy sub-felty to felty texture over the inoculum with broadly appressed and sodden texture over the greater part of the slant surface. The advancing zone was distinct and broadly appressed in all cases. In darkness at 22° C., the cultures exhibited faint zonations round the inoculum. On the 10th day after inoculation the mat remained thin, appressed and sodden with a felty inoculum. In 25-days-old cultures, the mat became a thin and powdery felt, this powdery appearance being less pronounced at room temperature. On *oat-meal-agar*, the texture of the superficial mycelium was like that described above, but the mat was more thick. At 22° C., the mat was

more loose. Colourless glistening drops of liquid appeared in old cultures in diffused light at 33° C. On *malt-extract-agar* and on *potato-starch-agar*, the same mat was presented but in the latter medium, growth was very poor.

(ii) *Colour.*—A tint of warm buff on *malt-extract-agar* and patches of chamois and cinnamon buff on *potato-starch-agar* only developed in 35-days-old cultures. No colour developed on other media, the cultures remaining white throughout.

(iii) *Rate of growth.*—The rate of growth was rather slow in comparison to other fungi. On *oat-meal-agar*, the rate of growth was rapid than on other media. Growth was best at 33° C., moderate at room temperature and comparatively less at 22° C. In general, growth was more rapid in darkness than in diffused light.

(iv) *Sporophore production.*—The wood-blocks were inoculated with 10-days-old mycelium on *potato-dextrose-agar*. The growth on the external surface of the blocks was poor and the mat was thin and slightly yellowish in colour. Tiny fruit-bodies developed in several cultures on agar media. A fruit-body, on section, showed basidia and basidiospores. The spores were oval and measured about $7 \cdot 8 \times 13\,\mu$.

(v) *Mycelium.*—Three main types of hyphæ can be distinguished in culture, *viz.*, (*a*) broad, thin-walled, hyaline, branched, closely septate, about $2 \cdot 7\,\mu$ broad : (*b*) narrow, thin-walled, hyaline, sparingly branched, septate, about $1 \cdot 3\,\mu$ broad and (*c*) thick-walled, sparingly branched, distantly septate, more or less coiled and $2 \cdot 7$–$4\,\mu$ broad. Chlamydospores, both terminal and intercalary, are present. The chlamydospores have thick walls showing striations, contain granular protoplasm and measure about $8\,\mu \times 5 \cdot 4\,\mu$ (Text-figs. 10–14).

5. Polystictus steinheilianus *Berk. and Lév.*

The fungus was identified by Bresadola and at first regarded as synonymous with *Trametes rigida* Mont. and Berk. but later considered as a good species. He also regarded it as synonymous with *Polystictus connexus* Lév.

Geographical distribution

The fungus occurs in Martinique, Brazil and Venezuela in S. America. It is also found in Bengal and Orissa in India.

Occurrence

It grows on dead logs of *Shorea robusta* Gaertn., *Tectona grandis* L. and dead bamboo stem.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose-agar*, the initial growth of the superficial mycelium produced a sub-felty to felty mat. The advancing zone at room temperature was narrow, appressed and sodden. No evident advancing zones could, however, be evident in other conditions. Faint zonations were noticed early in young cultures kept at 22° C. Growth seemed to be the best at 33° C.,

moderate at room temperature and comparatively less at 22° C. Vigorous growth at 33° C. was manifested by the rolling of the mycelium on the bare glass surface in 5-days-old cultures. In 10 days after inoculation, the mat invariably became felty with an appressed to sub-felty advancing zone. At 33° C., the upper advancing zone looked like a pile of felt and numerous glistening drops of liquid appeared towards the base of the tube. Rolling of the mat was evident in all the cultures. In old cultures, the felty mat gave place to a woolly growth, and a pile of felt at the upper advancing zone was prominent. On other media the sequence of texture as the cultures developed was like that described for *potato-dextrose-agar*.

(ii) *Colour.*—On *potato-dextrose-agar*, a tint of light buff to shades of isabella colour appeared in 5-days-old cultures under diffused light at 22° C. No colour developed in other conditions. After 10 days of inoculation, colouration appeared in diffused light at room temperature and in darkness at 22° C., and consisted of ochraceous tawny to buckthorn brown. The shades in diffused light at 22° C. depend to pinkish buff, cinnamon buff, tawny olive, Saccardo's umber and drab. The bare glass surface was stained due to tinting of the rolling mycelium. Thus, for this fungus pigmentation was best developed at 22° C. and least at room temperature. At 33° C. the colour appeared very late. Moreover in diffused light, pigmentation developed earlier than in darkness and also consisted of deeper shades. In 25 days, no new shades appeared, but the lighter shades were absent, while the deeper shades only persisted. Pigmentation was noticed at 33° C. In still old cultures, deeper shades consisting of light clay colour, deep sayal brown, tawny olive, etc., only persisted. On *oat-meal-agar*, the same shades of colour as described above were seen but colouration developed late. On *malt-extract-agar*, the shades were deeper than those seen for *potato-dextrose-agar*. The colour over pore-mouths consisted of shades of light cinnamon drab and light drab. These shades, later on, deepened to hair brown, pinkish buff, gull grey and deep gull grey. On *potato-starch-agar*, the intensity and variety of colours were best as compared to other media. Within 5 days after inoculation, pigmentation developed under all conditions and consisted of various shades of ochraceous tawny, cinnamon brown, olive buff, antimony yellow, yellow ochre, buckthorn brown and warm buff. Thus, the shades were much deeper as compared to other media. On the 10th day, the lighter shades deepened to dresden brown, tawny olive, clay colour, saccardo's umber, pinkish buff, cinnamon buff, sayal brown and mikado brown. Colour over pore-tubes consisted of shades of gull grey to deep gull grey. Intensity of colour seemed to be best developed under diffused light at 33° C. and at room temperature. At 22° C. comparatively less pigmentation was noticed, a fact quite contrary to that seen for the previous media.

(iii) *Rate of growth.*—The initial growth was very rapid and the slants were covered within a week in all cases except for *potato-starch-agar* where the growth was comparatively slow and the slants were covered within 10 days of inoculation. The rate of growth was, however, best at 33° C., moderate at room temperature and at 22° C.

The rate of growth in diffused light was more than in darkness under any conditions of temperature.

(iv) *Sporophore production.*—For the early development of fructification *malt-extract-agar* and *potato-starch-agar* proved to be the best. On *potato-dextrose-agar* and *oat-meal-agar* comparatively less number of tubes fructified. The fruit-bodies were resupinate and were mostly formed at the upper advancing zone. The pore-tubes were irregular and consisted of various shades of light cinnamon drab, light drab, hair brown, pinkish buff, gull grey and deep gull grey. The fruit-bodies were peculiar in that the hymenial surfaces after a time were covered by a fresh mycelial growth developed from the germination of the secondary spores. Bits of mycelium from 7-days-old cultures on *potato-dextrose-agar* were transferred to the wood-blocks of all the plants previously mentioned, in Roux-tubes and in flasks. Growth started within a fortnight and in about a month, most of the wood-blocks were thoroughly infected. The mycelium was seen condensing in patches. The colouration of the mycelium was however very slight. In about 2 months time, fruit-bodies developed in all the wood-blocks both in Rouxtubes as well as in flasks. A fruit-body, in section, showed the presence of hyphal pegs and secondary spores. On adding a drop or two of sterile distilled water aseptically to culture tubes, the same fruit-body showed many immature basidia.

(v) *Mycelium.*—The hyphæ in culture consists of three main types, *viz.*, (*a*) thin-walled, branched, hyaline, septate, with clamp-connections, about 2·7 μ broad; (*b*) thin-walled, rarely branched, hyaline, septate, without clamp-connections, about 2 μ broad, and (*c*) thick-walled, branched, without clamp connections and about 3 μ broad (Text-figs. 15–20).

6. Merulius similis *B. et Br.*

Geographical distribution

The fungus occurs in Portuguese Guinea, in Africa and Bengal, Assam (Lokra hills), United Provinces, Ceylon and Malaya Peninsula in Asia.

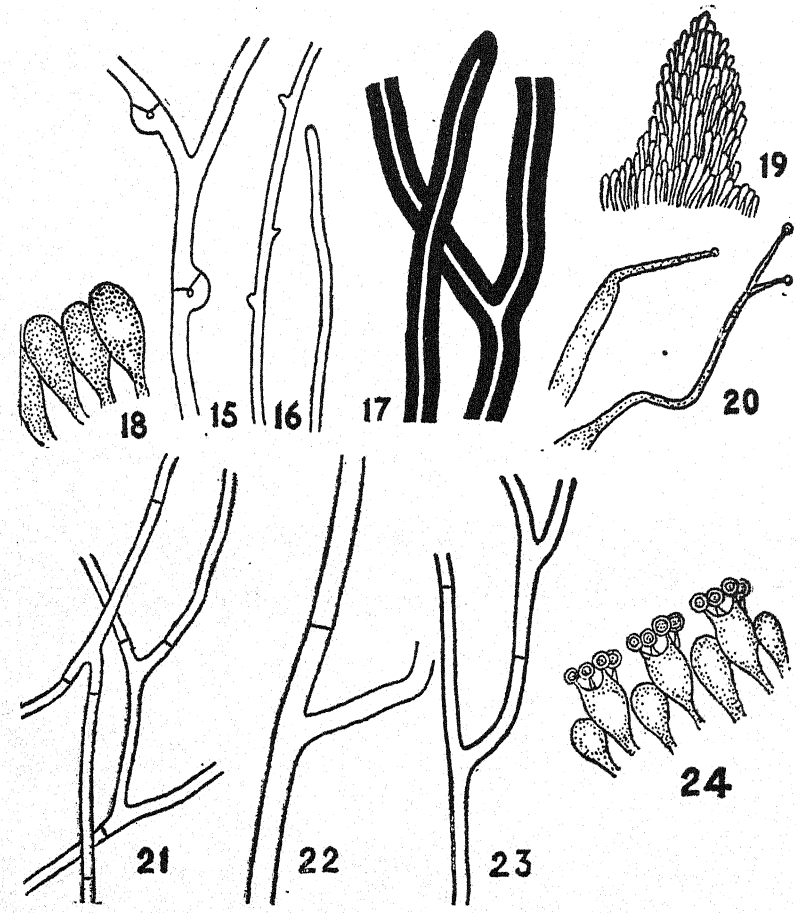
Occurrence

It grows in imbricate clusters on clumps and roots of living and dead bamboos and is very common in Calcutta and suburbs.

Fungus in culture

(i) *Habit of growth.*—On *potato-dextrose* and on *oat-meal-agar*, the initial growth of the superficial mycelium produced a felty mat with appressed advancing zones. The young cultures exhibited faint zonations at 22° C. as well as in room temperature. Within 10 days after inoculation the cultures became faintly granular due to irregular condensation of the mycelium. Rolling of the mycelium was only evident in darkness at 33° C. The cultures under different conditions of light and darkness exhibited no special distinguishing features. As the cultures became old, the mat became coarsely granular and eventually a compact and smooth felt resulted. Numerous colourless

glistening drops of liquid appeared in darkness at 33° C. and 22° C. These drops, later on, dried up. In darkness at 33° C., there was a pile of felt just behind the upper advancing zone. On the other media the mat remained as a thin felt.



Text-figs. 15–24—Figs. 15–20. *Polystictus steinheilianus:* 15. thin-walled, branched hypha with clamp-connections; 16. thin-walled, unbranched hyphæ without clamp-connections; 17. thick-walled hyphæ; 18. immature basidia; 19. hyphal peg; 20. secondary spores. Figs. 21–24. *Merulius similis:* 21. thin-walled, branched narrow hyphæ; 22. thin-walled, branched broad hypha; 23. thick-walled hypha; 24. basidia with basidiospores. (×520).

(ii) *Colour.*—On *potato-dextrose-agar* a shade of light buff developed in the cultures kept at 33° C. in about 10-days-old cultures. This deepened the pale ochraceous buff later on. The mat at the room temperature and at 22° C. remained chalk white throughout. On *oat-meal-agar*, shades of light buff, buff colour and pale ochraceous

buff developed in 10-days-old cultures only under diffused light at 33° C. Later on, these shades deepened to sea-shell pink and maize yellow. On other media, shades of buff, warm buff, mixed with white developed in old cultures at room temperature and at 33° C. under both conditions of light and darkness.

(iii) *Rate of growth.*—The rate of growth was moderately rapid on all the media, being best at 33° C., moderate at room temperature, and a little less at 22° C. Advance was more rapid in darkness than in diffused light.

(iv) *Sporophore production.*—Resupinate patches of fruit-bodies appeared on agar media in about 45 days. The wood-blocks were inoculated with mycelium from 10-days-old cultures on *potato-dextrose-agar*. Within a month the mycelium condensed at places forming a cushion with bright yellow colour. A fruit-body, on sectioning, showed the presence of basidia ($9 \cdot 75$–$10 \cdot 5\,\mu \times 7 \cdot 5$–$8 \cdot 25\,\mu$) with four strigmata and basidiospores ($3 \cdot 75\,\mu \times 3 \cdot 75\,\mu$).

(v) *Mycelium.*—Three main types of hyphæ can be recognised in cultures, *viz.*, (*a*) thin-walled, branched, septate, with granular contents, $1 \cdot 3$–$2 \cdot 7\,\mu$ broad; (*b*) thin-walled, branched, septate, with granular contents, $2 \cdot 7$–$4\,\mu$ broad, and (*c*) thick-walled, branched, hyaline and $1 \cdot 3$–$2 \cdot 7\,\mu$ broad. Calcium-oxalate crystals of various shapes are present in cultures (Text-figs. 21–24).

IV. General Considerations and Conclusions

An attempt has been made to summarise the influence of several external factors which affect the vegetative growth and fruit-body formation in artificial cultures of these fungi. The effect of a single factor has been studied by keeping the others constant.

(1) *Light*

The influence of light on Polypores has been fairly worked out. Long and Harsch[24] allowed direct sunlight to reach the young cultures for one to two hours, but later the amount of direct sunlight was decreased by light screens. This checked the mycelial growth and intensified the colours of the aerial mycelium. Fritz[19] carried on her investigations in complete darkness where the diagnostic characters are accurately manifested. In this investigation the cultures were placed both in diffused light and darkness. Direct sunlight was avoided as its unfavourable action has already been pointed out by Fritz.[19]

The effect of light on the growth and development of each of the fungi has already been described. In the presence of light, the mat becomes more compact due to early condensation and more rich and varied colouration is produced. The writers are of the opinion that although light causes early appearance of pigmentation, it in no way determines the final range of colours, which seems to be constant for each species when grown on a particular medium under definite temperature conditions. Cultures grown in darkness overrun rapidly on the surface of the agar slant.

(2) *Temperature*

Comparative cultural studies were made at ordinary room temperature (23° C.–27° C.) and at constant temperatures of 22° C. and 33° C. This is approximately the same as the range (22° C.–35° C.) used by Fritz.[19] Mounce[26] used a still lower temperature (0° C.–8° C.), which seems to be beyond the usual range.

In general, growth at 22° C. is very unsatisfactory, the mat being thin and loose. Faint zonation is noticed invariably in young cultures of all the fungi. The rate of growth is rather slow. Colour production is delayed and the intensity of pigmentation is much less than that in other temperature conditions. Mycelial growth seems to be best at 33° C., the mat being more compact due to early condensation. The rate of growth is also rapid, pigmentation appears earlier and more intense than in other conditions. At room temperature, the habit and rate of growth are moderate and the range of colour nearly approaches that found at 33° C. These observations agree with those of Fritz[19] and Mounce[26] who also noticed that intensification of colour takes place with increase in temperature. Since during the experiment no intermediate temperature between 27° C. and 33° C. was tried, the writers could not establish the optimum temperature at which growth was at its best but the temperature of 33° C. seemed to be fairly near the optimum.

(3) *Substratum*

(*a*) *Agar-media.*—Bose[12] and other workers found that *malt-extract-agar* was quite suitable for the display of cultural characters as well as for fruit-body formation. The authors, however, obtained better results on *potato-dextrose-agar* medium. Fritz[19] and Zeller[41,42] also found this medium quite suitable. Fritz[19] found that *potato-dextrose-agar* and *malt-extract-agar* were equally good but on the whole the former was more preferable, since mats were closely interwoven and as a consequence presented surfaces of more definite character. For this reason, she based her *key* by growth on *potato-dextrose-agar*. In diffused light and at a temperature of about 33° C., the medium provides the best condition for growth particularly in the case of Polypores. *Oat-meal-agar* medium proved satisfactory for all the fungi. *Potato-starch-agar*, on the whole, proved to be unsatisfactory except for *Polystictus steinheilianus* in which case colour production as well as development of fruit-bodies were found to be the best. Thus, a single medium has been found which is universally good for all fungi and gives a display of cultural characerts of all. Hence, for a comparative study of the cultural characteristics of fungi, it is always advisable to select a number of media, including, at least, one synthetic medium.

(*b*) *Wood-blocks.*—The wood-blocks were inoculated both in flasks and in Roux-tubes. The advantage of flask culture over Roux-tube culture is that in the former case, the mycelium has a good start for growth and attacks the wood earlier than in the latter. In Roux-tube cultures, the inoculum must include a sufficient amount of medium

so that the mycelium may retain its viability for a long time. It has been observed that the specific nature of the wood is not of much importance and growth takes place on all wood under proper conditions, since *Polystictus steinheilianus* fructified on *Bambusa arundinacea*, *Shorea robusta*, *Mangifera indica* and *Ceriops Roxburghiana*. The fact that no sporophore has ever been found in nature on any of these plants except *Shorea robusta* shows that there must be some factor present in the living wood which has been lost during the processes of drying. Another interesting observation was that *Polystictus steinheilianus* formed fruit-bodies on agar media within a week and thus had a remarkably short life-history. The same fungus, however, took two months to fructify on wood proving that life-histories of hard fungi are prolonged on wood-block cultures, as was pointed out by Bose.[13]

(4) *Effects of various factors on sporophore production*

It is known that the production of fruit-bodies in nature in many Hymenomycetes is more or less dependent upon the influence of light. Spaulding,[35] however, found a few Polypores fructifying in clay mines. Long and Harsch[24] observed that certain members of Polyporaceæ could produce fruit-bodies in complete darkness. In artificial cultures, so far as our observations indicate, sporophores were obtained as frequently in darkness as in light.

Detailed observations on the production of true pilei of *Polyporus brumalis* in artificial culture have been published elsewhere.[3] It was observed that the stalks of the fruit-bodies were positively phototropic but had no relation to the influence of gravity. The stalks, however, failed to produce typical pilei on agar media and various treatments of light, temperature and humidity showed that each of these conditions alone had very little effect on pileus-formation. Cultures were later grown on Badcock's medium[1] in Erlenmeyer flasks and it was observed that stalks were formed which reached the bases of the plugs where they remained as such without forming the pilei. As soon as the plugs were opened, the stalks elongated and formed normal pilei. Thus, it appeared that aeration was one of the main factors responsible for the production of normal pilei in *Polyporus brumalis*. In a recent paper Badcock[2] modified his earlier methods and thoroughly discussed the conditions influencing the development of normal fruit-bodies in culture. According to him the following conditions appear to be essential : the provision of a generous supply of a rich, well-aerated medium with plenty of moisture ; moderately high relative humidity, but not a saturated atmosphere, at the surface of the medium and around the developing sporophores ; and exposure to light of moderate intensity.

Sections through the hymenial surface of the fruit-bodies of *Polyporus brumalis*, *P. ochroleucus* and *Merulius similis* revealed the presence of basidia and basidiospores. *Polystictus steinheilianus* was remarkable in that the porous surface once formed was later masked by a fresh mycelial growth covering up the entire hymenial surface. This mycelial overgrowth was probably due to germination

of secondary spores which were abundantly found in the sections of such a fruit-body. Bose[13] maintains that a definite percentage of humidity is required for the production of basidia and basidiospores in the fruit-body in artificial culture but with the lowering of humidity, numerous secondary spores are produced. Sections through a part of the fruit-body of *Polystictus steinheilianus* in culture revealed the presence of secondary spores. But when few drops of sterile distilled water were poured aseptically into the culture tube, the same fruit-body, on section, revealed the presence of innumerable immature basidia.

That there is no relation between the appearance of glistening drops of liquid and the formation of fruit-body, was established during the course of the investigation. No doubt in many cases, fruit-bodies appeared immediately after the appearance of liquid drops, but this fact can, on no account, be taken as the cause. In cases of *Polyporus friabilis* and *Polyporus rubidus* glistening drops of liquid appeared and later on dried up, but in no case fruit-bodies developed.

A temperature of about 33° C. in diffused light seemed to be the optimum condition for fruit-body formation. At this temperature, the mat became compact and fructifications developed early.

(5) *Humidity*

To get spore-print from a dried sporophore, the method adopted by Bose[11] was followed. To maintain maximum humidity of the surrounding atmosphere during spore-discharge, moist chamber was made by lining the inside of a bell-jar with moistened blotting paper and placing some water inside the jar. That high humidity provided a favourable condition for vegetative growth was certain but how far the reproduction was retarded or accelerated could not be ascertained. In Roux-tubes, mycelial growth at the lower end of the wood-blocks near the water surface was vigorous and some of them travelled down to the water surface, grew luxuriantly and formed a dense floating mycelial mat. Fruit-bodies when formed on wood in Roux-tubes were seen at the portion of the blocks, away from the water surface.

(6) *Gravity*

The stalks of *Polyporus brumalis* did not respond to the force of gravity. The upward growth of the stalks was purely incidental for in culture tubes kept horizontally it was seen that the vertical stalks arising at the base of the tubes soon turned their apices and grew horizontally parallel to the glass surface.

SUMMARY

1. Six species of Bengal Polyporaceæ, namely, *Polyporus brumalis*, *P. friabilis*, *P. rubidus*, *P. ochroleucus*, *Polystictus steinheilianus* and *Merulius similis* have been studied.

2. The technique of making different types of cultures, preparation of media and the conditions under which the fungi were grown have been described.

3. The descriptions of the fungi including their geographical distribution, occurrence, habit, colour, rate of growth, mycelium in culture, sporophore-production on various media have been given.

4. General observations regarding the effects of light, temperature, substratum, humidity and aeration on vegetative growth and fruit-body formation have been made.

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LITERATURE CITED

1. Badcock, E. C. (1941) "New methods for the cultivation of wood-rotting fungi," *Trans. Brit. Mycol. Soc.*, **25**, 2, 200–05.
2. ——— (1943) .. "Methods for obtaining fructifications of wood-rotting fungi in culture," *ibid.*, **26**, 3–4, 127–32.
3. Banerji, S. N. and Bakshi, B. K. (1944) "On the production of true pilei of *Polyporus brumalis* (Pers.) Fr. in artificial culture," *Curr. Sci.*, **13**, 102–03.
4. Baxter, D. V. (1924) .. "*Fomes fraxineus* Fr. in culture," *Michigan Acad. Sci.*, **4**, 55–66.
5. Berkeley, M. J. (1839) "Description of exotic fungi in the collection of Sir W. J. Hooker, from memoirs and notes of J. F. Klotzsch, with additions and corrections," *Ann. Nat. Hist.* **3**, 375–401.
6. Bose, S. R. (1920) .. "Polyporaceæ of Bengal, III," *Bull. Car. Med. Coll.*, **1**.
7. ——— (1921) .. "Polyporaceæ of Bengal, IV," *ibid.*, **2**, 1–5.
8. ——— (1928) .. "Polyporaceæ of Bengal, IX," *Jour. Dept. Sci. Cal. Univ.*, **9**.
9. ——— (1934) .. "Polyporaceæ of Bengal, X," *ibid.*, **11**.
10. ——— (1937) .. "Polyporaceæ from Lokra Hills (Assam)," *Ann. Mycol.*, **35**.
11. ——— (1929) .. "Revival of an old fruit-body of *Hexagonia discopoda*, Pat. and Hariot, and successful spore-culture from its fresh spore-discharge," *ibid.*, **27**, 321–23.
12. ——— (1930) .. "Biology of wood-rotting fungi common in forest areas," *Jour. Linn. Soc.*, **48**, 417–38.
13. ——— (May 1940) "Moisture relation as a determining factor in the transformation of the basidia of certain Polyporaceæ," *Nature*.
14. Bresadola, G. (1920) .. "Selecta mycologica. I. Diagnoses specierum novarum," *Ann. Mycol.*, **18**, 26–58.
15. Buller, A. H. R. (1906) "The biology of *Polyporus squamosus* Huds.," *Jour. Econ. Biol.*, **1**, pt. 3, 101–38.
16. ——— (1922, 1924) *Researches on Fungi* (Vols. II and III).
17. Butler, E. J., and Bisby, G. R. (1931) *The Fungi of India*, Scientific Monograph No. 1, I. C. A. R., Delhi.
18. Duggar, B. M., Severy, J. W., and Schmitz, H. (1917) "Studies in the physiology of the fungi. IV. The growth of certain fungi in plant decoctions," *Ann. Miss. Bot. Gar.*, **4** (2), 165–73.

19. Fritz, C. W. (1923) .. "Cultural criteria for the distinction of wood-destroying fungi," *Trans. Roy. Soc. of Canada*, 3rd Ser., **17**.

20. Hartig, R. (1878) .. *Zersetzungserscheinungen des Holzes der Nadelholzbaume und der Eiche.*

21. ——— (1900) .. *Lehrbuch der Pflanzenkrankheiten.*

22. Lloyd, C. G. (1904–19) *Mycological Letters*, 1–69 (each separately paged), Cincinnati, Ohio.

23. ——— (1898–1925) *Mycological Notes*, 1–75, 1–1364, Cincinnati, Ohio.

24. Long, W. H., and Harsch, R. M. (1918) "Pure cultures of Wood-rotting Fungi on artificial media," *Jour. Agr. Res.*, **12**, No. 2.

25. Lyman, G. R. (1907) .. "Culture-studies on Polymorphism of Hymenomycetes," *Proc. of the Boston Soc. of Nat. Hist.*, **33**, 125–210.

26. Mounce, Irene (1929) "Studies in forest pathology. II. The Biology of *Fomes pinicola* (SW.) Cooke," *Dominion of Canada, Dept. of Agriculture, Bull.* 111, New series, Ottawa.

27. Mundkur, B. B. (1938) *Fungi of India*, Supplement I, Scientific Monograph No. 12, Imp. Coun. Agr. Res. Delhi.

28. Oudemans, C. A. J. A. (1919) *Enumeratio systematica fungorum.*

29. Petch, T. (1916) .. "A preliminary list of Ceylon polypori," *Ann. Royal Bot. Gard. Peradeniya*, **6**, 1–58.

30. Rabenhorst (1884) .. *Kryptogamen Flora*, I.

31. Rhoads, A. (1918) .. "The biology of *Polyporus pergamenus* Fr.," *Tech. Pub. N.Y.S. Coll. For.*, **11**, 1–197.

32. Ridgeway, R. (1912) .. *Colour Standard and Colour Nomenclature*, Washington.

33. Saccardo, P. A. (1888) *Sylloge fungorum.*

34. Spaulding, P. (1905) .. "Cultures of wood-inhabiting fungi," *Sci.*, **21**, 143.

35. ——— (1910) .. "Fungi of clay mines," *Rep. Mo. Bot. Gard.*, **21**, 189–195.

36. Theissen, F. (1911) .. "Fungi aliquot Bombayenses a Rev. Ed. Blatter collec." *Ann. Myc.*, **9**, 153–59.

37. von Schrenk, H. (1900) "Some diseases of New England Conifers," *U.S. Dept. Agri. Div. Veg. Phys. Bull.*, **25**.

38. ——— (1900) .. "Two diseases of red cedar caused by *Polyporus juniperinus* n.sp. and *Polyporus carneus* Nees.," *U.S. Dept. Agri. Div. Veg. Phys. and Path. Bull.*, 21.

39. Ward, H. M. (1897) .. "On the biology of *Stereum hirsutum*," *Phil. Trans. Roy. Soc. London*, **B 189**, 123–34.

40. White, J. H. (1919) .. "On the biology of *Fomes applanatus* (Pers.) Wallr.," *Trans. Roy. Can. Inst.*, **12**, 133–74.

41. Zeller, S. M. (1916) .. "Studies on the Physiology of the Fungi. II. *Lenzites sæpiaria* Fries, with special reference to enzyme activity," *Ann. Mo. Bot. Gard.*, III, 439–512.

42. ——— (1917) .. "Studies on the Physiology of the Fungi III. Physical properties of wood in relation to decay induced by *Lenzites sæpiaria* Fries," *ibid.*, IV, 93–155.

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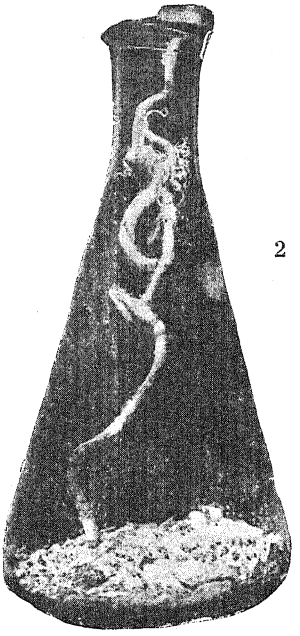

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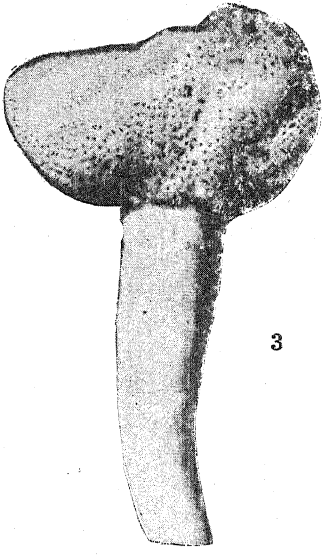

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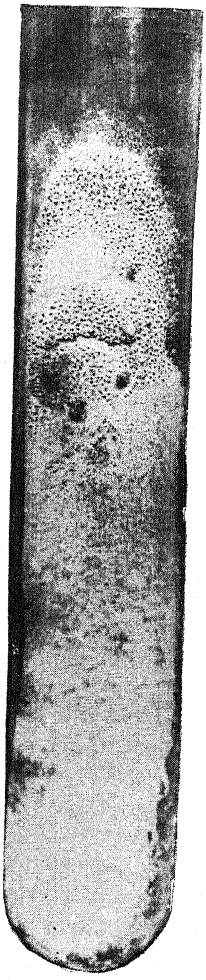
SACHINDRANATH BANERJEE AND BIMAL KUMAR BAKSHI—

STUDIES IN THE BIOLOGY OF WOOD-ROTTING FUNGI OF BENGAL



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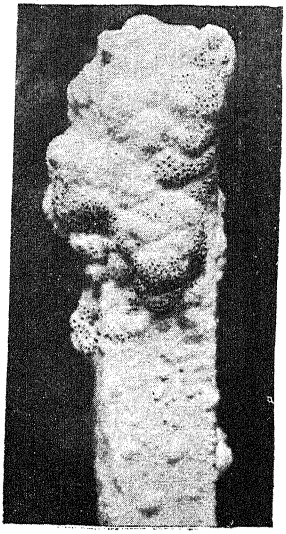

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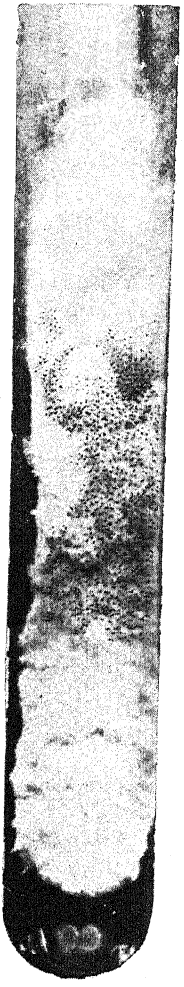

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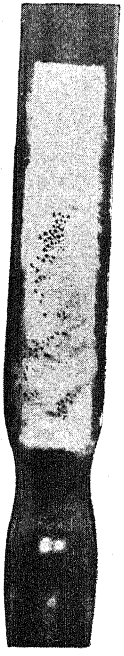

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SACHINDRANATH BANERJEE AND BIMAL KUMAR BAKSHI—

STUDIES IN THE BIOLOGY OF WOODR-OTTING FUNGI OF BENGAL

EXPLANATION OF PLATES

PLATE II

Fig. 1. Stalks of fruit-bodies of *Polyporus brumalis* formed on potato-dextrose-agar about 40-days-old (×3).

Fig. 2. A fruit-body of *P. brumalis* showing typical pileus formed on Badcock's medium (½ Nat. size).

Fig. 3. A pileus of *P. brumalis* showing hymenial surface with well-developed pores on Badcock's medium (×3).

PLATE III

Fig. 4. A resupinate fruit-body of *Merulius similis* formed on Potato-dextrose agar about 30-days-old (Nat. size).

Fig. 5. A resupinate fructification of *Polystictus steinheilianus* formed on malt-extract-agar about 20-days-old (Nat. size).

Fig. 6. A fruit-body of *P. steinheilianus* formed on potato-dextrose-agar about 15-days-old (½ Nat. size).

Fig. 7. A fruit-body of *P. stinheilianus* formed on sterilized wood block of *Mangifera indica* about 2-months-old (½ Nat. size).

Fig. 8. An irregular resupinate fruit-body of *P. steinheilianus* formed on a sterlised wood-block of *Shorea robusta*, about 2-months-old (Nat. size).

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SOME FOSSIL LEAVES OF *LITSAEA LANUGINOSA* NEES. FROM THE KAREWA BEDS AT LIDDARMARG, PIR PANJAL, KASHMIR

BY G. S. PURI

Department of Botany, University of Lucknow

Received for publication on May 18, 1945

INTRODUCTION

IN the year 1911, Mr. C. S. Middlemiss of the Geological Survey of India during his geological studies in Kashmir, made a collection of about 200 specimens of fossil plants from Liddarmarg, a small village situated on the densely forested northern slopes of the Pir Panjal Range, which rises on the southern side of the valley in a series of high mountain chains.

The fossiliferous beds, which have yielded a fossil flora of 30 genera and 40 species (Puri, 1941, pp. 7–8) belong to the Lower Karewa formations. These deposits on recent geological evidence, are considered to be Lower Pleistocene in age. At first they were considered to form a part of the Upper Karewas (see de Terra and Wodehouse, 1935, p. 5) which are of the Upper Pleistocene Age, but later on de Terra (see de Terra and Paterson, 1939, pl. 55 ; pp. 109–114) changed his views in favour of the Lower Pleistocene Age for these beds. This view was long ago hinted at by Middlemiss (1911), when he referred the fossil beds at Liddarmarg as "Older Karewas".

The plant-bearing outcrops occur at an altitude of 10,600 ft. above sea-level and, as Middlemiss (*loc cit.*, pp. 121–22) writes, "are exposed in two stream beds near the present Gujar (herdsmen) encampment of Liddarmarg (lat. 38° 48′ ; long. 74° 39′). The dip is of the usual slightly inclined character, and the beds were found to contain a flora of well preserved leaves." This collection was examined by Middlemiss who, with the help of Mr. I. H. Burkill, was able to identify most of the fossil

leaves and announced (*loc. cit.*, p. 122) the occurrence, among the collections, of a few leaves of *Cinnamomum*, one of which according to them was specifically identical with *C. Tamala* Nees., a moderate-sized tree of the tropical and sub-tropical Himalayas.

The entire collection of Middlemiss, together with the original identifications, was kindly lent to me in 1939 by the Director, Geological Survey of India, for study at Lucknow under the guidance of Professor B. Sahni, F.R.S. On my examination of this material I find that a large number of the fossil leaves were unnamed and most of the original identifications of the named specimens the labels of which were fortunately preserved with the fossils, on comparison with my identifications have proved to be erroneous. A comprehensive description of the fossil flora discovered in this material, and another collection made in 1932 by Dr. H. de Terra from a different spot in the same locality, will be published in a series of papers at a later date. But a short note dealing with the description of hitherto unfigured and incorrectly identified leaves supposed to be of "*Cinnamomum Tamala*", but which are identical in all respects with living leaves of *Litsæa lanuginosa* Nees., is given in the following few pages.

Description

Phylum	..	Angiospermæ.
Subphylum	..	Dicotyledonæ.
Division	..	Archichlamydeæ.
Order	..	Laurales.
Family	..	Lauraceæ.
Genus	..	*Litsæa.*

Litsæa lanuginosa Nees.

(Pl. IV, Figs. 1–4)

The leaf fragments described below are poorly preserved impressions embedded in a fine-grained, compactly set clays, which are mostly greyish black in colour. The clays are composed of irregularly bedded layers, several inches thick, and it is, therefore, rarely possible to recover a complete leaf by splitting bigger blocks along the plane of bedding.

The entire collection before coming into my hands was heavily coated with some preservatives applied with a view to preserve the specimens as Middlemiss (*loc. cit.*, p. 122) wrote "there by painting them at Mr. Blyth's suggestion, with gelatine first and canada-balsam varnish afterwards, I was able to preserve a large number showing all the delicate venation, serrated edges, and in one or two instances even the deep red tints of the original fallen leaves".

Plate IV, Figs. 1–3 show natural size photographs of three leaf fragments, which represent the basal half of leaves, the apical parts being entirely missing in all. The shape of the fossil leaves cannot be definitely ascertained on account of their fragmentary nature but by comparing them with living leaves of this species it seems probable

that they had oblong-lanceolate lamina. The fragments show a slight variation in size. The lamina measures 2·5″ long by 1·1″ in the broadest part in the smallest specimen (Fig. 1) and it is 2·5″ × 1·4″ in Fig. 2. In the largest fragment (Fig. 3) it is 4·85″ × 1·9″. By a comparison of the fossil fragments with living leaves it is suggested that the broadest part in a fragment is probably the middle part of the lamina from which it gradually narrows down into a wedge-shaped base seen prominently in Fig. 2. The base in Fig. 3 is slightly distorted and curved to one size, due probably to a break in this region of the leaf, which might have been caused before or at the time of its deposition. In this specimen a bit of the petiole is also preserved. The margins are entire ; the apex is broken in all specimens and cannot be definitely ascertained but it seems to have been acute as in living leaves.

The venation is apparently pinnate but a closer examination reveals it to be strongly sub-triplinerved at the base. A strong midrib, which has left a fairly deep groove in Figs. 1, 2 and stands out in the form of a ridge in Fig. 3, runs in the lamina dividing it into slightly unequal halves. A comparison of the fossils with living leaves, in which the midrib is marked out on the lower surface, it is suggested that the photographs 1 and 2 are impressions from the lower surface and Fig. 3 is from the upper surface of the leaf. Two or three pairs of prominent secondaries arise in the upper part of the fragments at acute angles, in an alternate manner, on either side of the midrib. They follow a straight course in the lamina and are ending abruptly in the margins. The basal pair of secondaries, which is about half as thick as the midrib, arises from the midrib at very acute angles a short distance above the base and runs as far as middle of the lamina and finally ends in the margins far higher up from its point of origin. In one specimen (Fig. 2) a small secondary rib arises very close to the base on one side of the midrib and follows a straight course in the lamina. The secondaries in one specimen (Fig. 1) are quite close to one another but they are fairly wide apart in other specimens. The second basal lateral on the left side in Fig. 2 arises at a distance of 1·2″ from the basal pair. Tertiary and finer reticulations being poorly preserved in all specimens are not brought out in any of the photographs.

The fossils were confused and referred to *Cinnamomum Tamala* Nees., by Middlemiss and Burkill who failed to notice the differences in the details of venation between the two. Plate IV, Fig. 5 is a natural size photograph of a living leaf of *C. Tamala* which is given here to show contrast in venation with fossils and living leaf of *L. lanuginosa* (Pl. IV, Fig. 4). In addition to the basal pair of secondaries present in the fossils as well as in living leaf of *C. Tamala*, the former have two to three additional pairs of laterals, which are altogether absent in the latter. Each of the two basal secondaries in *C. Tamala* arises from the midrib at acute angles and runs in the lamina, as far up as the apex, making an arch parallel to the margin the convexity of the arch facing outwards and finally ends near the apex. In the fossils the basal laterals while running up do not make arches but follow a straight course as far as middle of the leaf and finally end in

the margins about half-way from the base. In details of venation, shape, size, margins, etc., our fossil fragments are identical with living leaves of *Litsæa lanuginosa* Nees. (see Fig. 4), a tropical tree of the sub-Himalayan regions.

Number of specimens.—Three.

Occurrence.—Liddarmarg at 10,600 ft., in the Pir Panjal Range, Kashmir.

Collector.—C. S. Middlemiss, 1910.

Registered Nos. of figured specimens.—

Pl. I, Fig. 1 : G.S.I. No. K 14/951 (II).
Pl. I, Fig. 2 : G.S.I. No. K 14/948a27.
Pl. I, Fig. 3 : G.S.I. No. K 14/951 (I).

Modern Distribution of *Litsæa*

The genus *Litsæa* is at the present time confined mainly to the tropical regions of Asia and also occurs rarely in boreal parts of America. In Asia, it occurs in the Himalayas, Burma and extends in the south-easterly direction through Malaya Peninsula and Malaya Islands, into tropical and subtropical forests of Australia, New Zealand and New Caledonia. It also spreads northwards from the Himalayas and occurs commonly in North as well as South China, extending as far north as Japan. Towards the south it spreads into Ceylon.

Modern Distribution of the Fossil Species

Litsæa lanuginosa, which belongs to the section *Neolitsea* of the genus, is definitely tropical in its modern distribution and occurs in India in the outer Himalayan ranges fairly scattered from Kashmir to Sikkim. It is extremely rare in the north and north-western outer Himalayas and according to Parker (1918, p. 430), who is considered to be an authority on the forest botany of these mountains, the species is absent from the Sutlej westwards. However, an authentic sheet in the Herbarium of the Forest Research Institute, Dehra Dun, shows that the species was collected from Muzaffarabad in Kashmir at an altitude of 4,000 ft. Eastwards, it occurs sparingly in sheltered places between the altitudes of 2,000 and 4,000 ft. above sea-level. It has been collected at Suni along Sutlej river and is also recorded from Simla District near Kalka. Further eastwards, it ascends to higher altitudes and has been collected from Rispana Valley, Mussoorie, at 5,800 ft. and occurs at 6,000 ft. above sea-level in the Sikkim Himalayas. It has been collected in this region from several places in Garhwal, Naini Tal, and Nepal, growing in shady places or occupying cooler aspects of these hills. The species is recorded from as far east as Khasi Hills and grows at an altitude of 8,000 ft. in Manipur, Assam. So far as we know, it has never been found growing or collected from Kashmir Valley, the northern slopes of the Pir Panjal Range, the southern slopes of the Main Himalayas, Murree Hills or the Kagan Valley, and its absence at the present time from all parts of Kashmir, excepting Muzaffarabad, and neighbouring mountain ranges is striking. Although our information

regarding data on the modern distribution of the species is based on literature and authentic sheets in the Herbaria yet its absence from these regions is not improbable and seems to conform to the prevailing climatic conditions in this part of the Himalayas.

Conclusions

The present discovery of *Litsæa lanuginosa*, a tropical species of the Lauraceæ, from Liddarmarg at an altitude of 10,600 ft., furnishes further data in favour of the theory of the Himalayan uplift during the Pleistocene. Its occurrence in the fossil beds at such high altitudes, where it has never been found growing at the present time, is a direct proof to show that the plant-bearing beds have been uplifted since the time *Litsæa lanuginosa* lived in the valley, which must have had a tropical climate at that time. In fact the prevalence of a tropical climate in the Kashmir Valley during Lower Pleistocene times has already been conclusively proved on palæobotanical evidence by the author (1943) and the present discovery lends further support to the idea of an extension of tropical and sub-tropical montane forests in the Kashmir Valley, from where they have now disappeared on account of the changed climatic conditions brought about by the uplift of the Pir Panjal Range.

The incorporation of the present species in the Liddarmarg flora in place of *Cinnamomum Tamala* has not materially altered the general features of the flora, which still continues to indicate a tropical climate as before.

Summary

The paper describes some fossil leaves of *Litsæa lanuginosa* Nees., a tropical species of the Lauraceæ (wrongly referred by I. H. Burkill and Middlemiss to *Cinnamomum Tamala* Nees.) from C. S. Middlemiss's collection of 1910 from the Lower Karewa beds (Pleistocene), which are exposed along two streams near Liddarmarg (alt. 10,600 ft., Pir Panjal Range, Kashmir Valley). The fossils were lent to the author for study by the Director, Geological Survey of India.

L. lanuginosa is confined at the present time to the tropical parts of the Outer Himalayas from the Sutlej eastwards between the altitudes of 2–4,000 ft., but it ascends to the altitude of 5,800 ft. in Rispana Valley, Mussoorie. Further east it occurs in Garhwal and Nepal, and ascends to 6,000 ft. in Sikkim. It is recorded from as far east as Assam in the Khasi Hills and has been collected from as high an altitude as 8,000 ft. from Manipur. It is conspicuous by its absence from North and North-Western Himalayas and does not grow anywhere in Kashmir excepting Muzzaffarabad at 4,000 ft. It is also absent from the neighbouring regions including Pir Panjal Range, the Kagan Valley and the Murree Hills.

This sharp contrast in the past and present distribution of the species in Kashmir lends additional support to the theory of the Pleistocene uplift of the Himalayas, already confirmed by the author on palæobotanical evidence. The idea of an extended occurrence of

a tropical and sub-tropical forest flora into the Kashmir Valley across the Pir Panjal Range during the Lower Pleistocene times thus gains further ground.

This incorporation of *Litsæa lanuginosa* in the fossil flora in place of *Cinnamomum Tamala* does not materially change the general feature of the flora.

ACKNOWLEDGMENTS

I am highly indebted to Prof. B. Sahni, F.R.S., for his guidance and criticism during the preparation of this paper. I also take this opportunity of thanking the Director, Geological Survey of India, for the loan of the material and photographs of the fossils. The investigations of the Karewa flora at Lucknow have been financed by research grants from the University of the Panjab and a Research Fellowship from the University of Lucknow for which the author wishes to thank the Vice-Chancellor of the Panjab University, Principal Jodh Singh of the Khalsa College, Amritsar, and authorities of the Lucknow University. Living leaves of *Cinnamomum Tamala* were kindly sent to me at my request by the Curator, Botanical Gardens, Sibpur, to whom my best thanks are also due.

LITERATURE CITED

De Terra, H. and R. P. Wodehouse (1935) .. "Pleistocene Pollen of Kashmir," *Mem. Conn. Acad.*, **9**, Art. 1.

——— and T. T. Paterson (1939) "Studies on the Ice Age in India and associated Human Cultures," Carnegie Institution, Washington.

Middlemiss, C. S. (1911) .. "Sections in the Pir Panjal Range and Sind Valley, Kashmir," *Rec. Geol. Surv. Ind.*. **41.**

Parker, R. N. (1918) .. "A forest flora for the Punjab with Hazara and Delhi," Lahore, 430.

Puri, G. S. (1941) .. "Palæobotany in India, Progress Report for 1940." *Jour. Ind. Bot. Soc.*, **20**, 7–8.

——— (1943) .. "The occurrence of *Woodfordia fruticosa* (Linn.) S. Kurz. in the Karewa deposits of Kashmir, with remarks on changes of altitude and climate during the Pleistocene," *ibid.*, **22**, 125–31.

EXPLANATION OF PLATE IV

The figures are from untouched negatives and are of natural size.

Litsæa lanuginosa Nees.

Fig. 1. Fossil leaf fragment (impression of the lower surface).

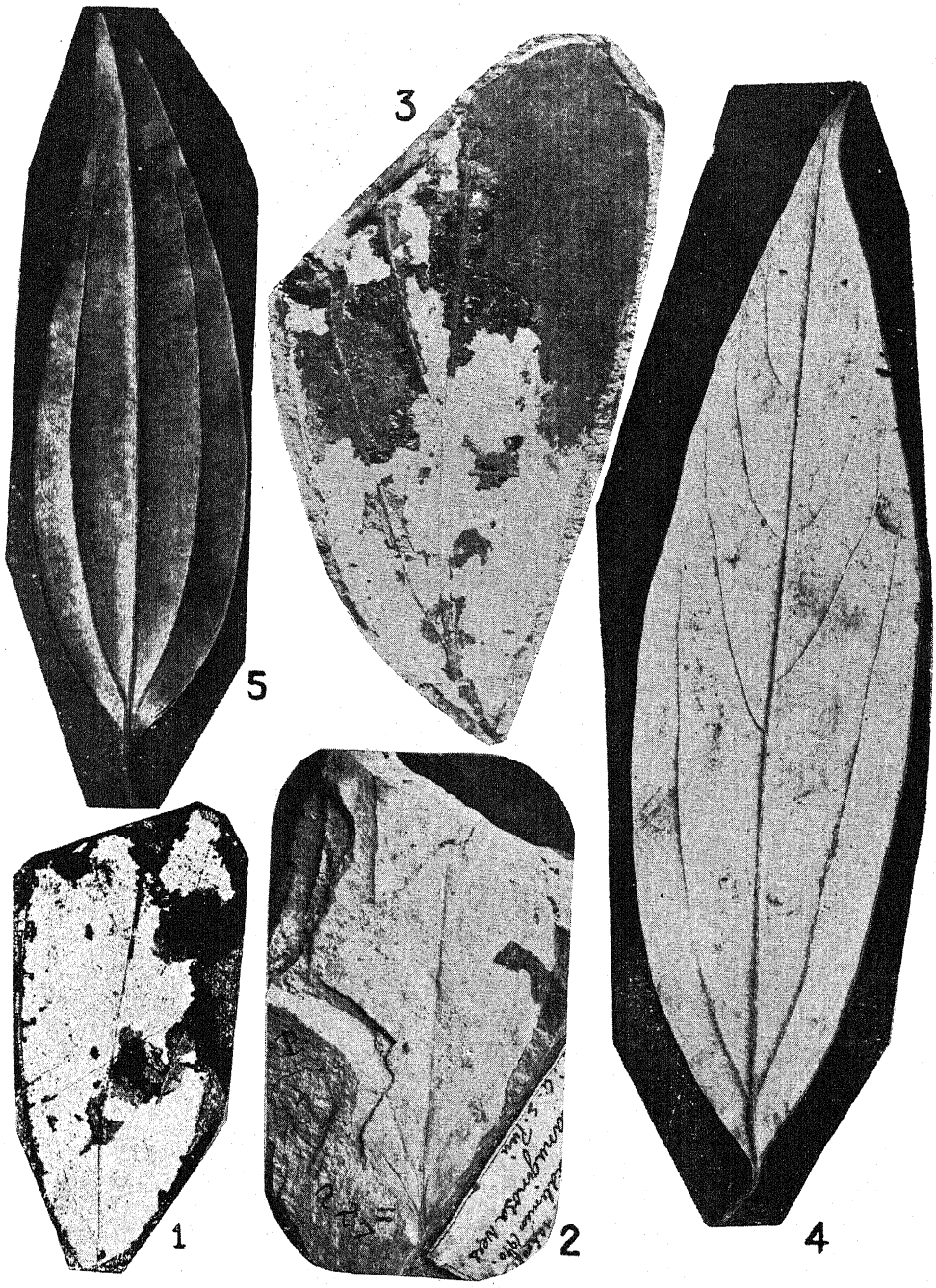
Fig. 2. Fossil leaf fragment (impression of the upper surface).

Fig. 3. Fossil leaf fragment (impression of the lower surface).

Fig. 4. Modern leaf for comparison with the fossils and contrast with modern leaf of *Cinnamomum Tamala* Nees.

Cinnamomum Tamala Nees.

Fig. 5. Modern leaf to show contrast in venation with the fossil fragments and modern leaf of *Litsæa lanuginosa* Nees.



G. S. PURI—

SOME FOSSIL LEAVES OF LITSÆA LANUGINOSA *NEES. FROM THE KAREWA BEDS AT LIDDARMARG, PIR PANJAL, KASHMIR*

STUDIES IN THE DISEASES OF *MANGIFERA INDICA* LINN.

Part V. On the Die-back Disease of the Mango Tree

BY S. N. DAS GUPTA AND A. T. ZACHARIAH (MISS)

Department of Botany, Lucknow University

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INTRODUCTION

MANGO trees are subject to a disease in which the leaves fall off, twigs dry up, and the entire branch or the part affected assumes an appearance of dry, dead, leafless twigs sticking up from among the green healthy foliage resembling the 'die-back' and twig blight of trees commonly described in pathological literature. The disease is prevalent not only in the U.P. but also in other mango-growing provinces of India. A number of diseased twigs were collected from Lucknow and Madras, and the investigation was undertaken to ascertain the cause of the disease. The results are presented in this paper. A preliminary note was published earlier (Das Gupta and Zachariah, 1939).

SYMPTOMS

General.—The effect of the disease on the general appearance of the trees is noticeable at anytime of the year ; but it is most conspicuous during the months of September and October. A large number of wilted branches and twigs are prominent among the green foliage of infected trees. In advanced stages of the disease leafless branches as well as twigs showing different stages of wilt give the tree an appearance as if it has been scorched by fire (Pl. V, Fig. 1). A magnified view of a portion of tree affected by the disease is shown in Pl. V, Fig. 2. Generally the smaller twigs and branches of comparatively old trees are affected.

External.—The first external evidence of the disease is the discolouring and darkening of the bark at a certain distance from the tip (Pl. V, Fig. 3*a*). The front and side views of the dark infected area are shown in Pl. V, Figs. 8 and 9. Such dark patches usually occur in the young green twigs and are hardly distinguishable in older branches.

As the darkening advances towards the tip the leaves just above the infected region wither (Pl. V, Fig. 3*b*). The upper leaves lose their healthy green colour and gradually turn brown (Pl. V, Fig. 4*b*). The browning starts at the base of the leaf, spreads along the midrib, and then out along the veins to the margine. This is followed by the browning of the whole leaf accompanied by the upward rolling of the margine (Pl. V, Fig. 5*a*). Eventually the affected twig or branch

dies and shrivels up (Pl. V, Fig. 4). A magnified view of the rolled shrivelled leaves is shown in Pl. V, Fig. 6. The brown rolled leaves often persist for a month or more (Pl. V, Fig. 7) and finally drop off, leaving the shrivelled twig altogether bare of leaves which is the characteristic of the advanced stage of the disease (Pl. V, Fig. 7*b*). Infection may be accompanied by the extrusion of gum (Pl. V, Fig. 9*j*).

HISTOPATHOLOGY

The infected twig shows an internal discolouration which is found to extend at an earlier stage of the disease about an inch on either side, towards the tip and the base of the twig, beyond the external darkened bark. The diseased twigs when cut out slantingly along the long axis through the infected region reveal a brown streaking of the vascular tissues, namely, cambium and phloem (Pl. V, Fig. 10). The internal discolouration is diffuse and uniform and appears as a dark streak between the stele and the cortex (Pl. V, Fig. 11). Series of sections of affected twigs showing different stages of the disease were examined to study the effect of the disease on the internal tissues.

Sections at about four inches below the growing point of a twig at a very early stage of infection which appeared healthy except for a short discoloured area on the stem showed slightly shrivelled, epidermal and sub-epidermal cells. The internal discolouration was manifested by the browning of certain regions of the cambium and phloem, where some of the cells were found to be plugged with a yellow gum-like substance. A few hyphæ were seen in the xylem vessels. The inner regions of the cortex appeared unaffected while the cells of the outer layers had started shrivelling.

In very advanced stages of the disease, the cells of the different tissues of the stem were badly shrivelled. The xylem vessels were plugged with fungal mycelium. The stele and the outer layers got separated from each other along the discoloured band at the cambial region where the cells had disintegrated. Numerous hyphæ were found in this region. A few hyphæ were also found in the cells of the cortex. The mycelium was found not only in the bundles of the stem, but also in the petioles and midribs of leaves of infected twigs.

EXPERIMENTAL

Infected twigs were collected from trees of the Botany Department, Lucknow University, and the Isabella Thoburn College Orchard, Lucknow. Material from Madras was collected by Dr. T. S. Sadasivan from trees showing variable symptoms of die-back.

Twigs showing different stages of the disease were chosen for the investigation. In some the leaves were just turning brown ; in others they had completely shrivelled up. In very advanced stages the twigs were quite dry with no leaves on them. The surface of the bark showed leaf-scars, lenticels and breaks caused by natural cracking of the outer layers.

As isolation from infected twigs by taking small inocula from the internal diseased tissue and directly culturing them in nutrient

medium proved impracticable, the diseased twigs were first placed in moist chambers and the hyphæ growing out from the pieces were sub-cultured in the standard synthetic medium.

The infected twigs were first cleaned by swabbing with cotton-wool dipped in a saturated solution of borax. They were, then, cut into small pieces, 1–1½ inches long and each piece was separately washed in a saturated borax solution, steeped in 0·1% mercuric chloride for about five minutes, shaking vigorously at intervals and was finally washed in three changes of sterile distilled water. After this surface sterilisation, the twig pieces were placed in sterile moist chambers. A large number of twig pieces were tried with wood and bark intact, while other pieces had the bark stripped off from the wood portion and placed in separate moist chambers. Within 3–5 days these twig pieces showed a profuse growth of hyphæ in them. On an external examination only two types of hyphæ could be distinguished, one being pure white, the other whitish in colour at the beginning and turning dark with age.

Separate inoculations were made with hyphæ arising from different parts of the twigs pieces namely cut ends, surface of wood, inner and outer surfaces of bark, on standard synthetic medium and malt agar. By this method altogether five fungi were isolated, *viz.*, *Botryodiplodia theobromæ* (B), *Phoma* (P), *Fusarium* (F), *Fusarium* (f), *Pestalozzia* (Pz), *Alternaria* (A).

The results are shown in Table I.

TABLE I

Locality	Twig 1			Twig 2			Twig 3			Twig 4			Twig 5			Twig 6		
	b&w	b	w	b&w	b	w	b&w	b	w	b&w	b	w	b&w	b	w	b&w	b	w
Botany Department	B	B	B	BF	BF	B	PF	PF	F	P	fA	P	P	Pz,f	P	PF	fA	F
Isabella Thoburn College	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Madras	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

b & w=bark and wood, b=bark, w=wood.

On examination of the result it was found that all the six twigs belonging to different trees of the Isabella Thoburn College Orchard and those belonging to Madras showed the presence of *Botryodiplodia theobromæ* alone both from bark and wood whereas the twigs from the Botany Department garden gave anomalous results. The twig 1 produced only *Botryodiplodia theobromæ* from all its parts, the twig 2 produced *Botryodiplodia theobromæ* from wood only, but in bark, and bark and wood *Fusarium* was associated with *Botryodiplodia theobromæ*. Twigs 3, 4, 5 and 6 were characterised by the total absence of *Botryodiplodia theobromæ*, instead of which were found four different fungi,

Phoma, Fusarium, Alternaria and *Pestalozzia* either singly or variously associated. The last three were invariably associated with bark. In each case, however, the wood portion produced only one kind of fungus, for example, *Fusarium* in the wood of twigs 3 and 6 and *Phoma* in that of twigs 4 and 5.

It was apparent from this preliminary experiment that so far as the twigs from Isabella Thoburn College and those from Madras were concerned, the disease was due to *Botryodiplodia theobromæ*. But the appearance of more than one fungi from the wood of different diseased twigs of the mango tree of the Botany Department indicated that each of these fungi may be a potential parasite causing the disease.

Detailed work was next undertaken to confirm the results obtained : (*a*) by studying the spatial distribution of the fungal strains in diseased twigs, and (*b*) by inoculation experiments.

Spatial distribution of fungi.—The same experimental method as described before was employed. Twigs showing various stages of the disease and collected from localities already mentioned were surface sterilized. The diseased, and in some cases, apparently healthy parts were then divided into pieces 1–1½ inches in length (whenever possible bark was separated from wood) and put in moist chamber. The spatial distribution of the fungi in the twigs was found out by noting and drawing the exact position of the piece on the twig and numbering the successive pieces from the tip towards the base. A few leaves of each twig, too, were put in moist chamber after surface sterilisation. The fungi appearing from the twig pieces and leaves were inoculated on standard synthetic medium.

Although a very careful record was made of all the fungi which appeared from bark, and wood of different twigs tested, it was obvious that only the fungi growing out of the wood region, would be responsible for the disease. The fungi arising from bark or the surface of the wood would provide with subsidiary evidence regarding the causal organism. While analysing the results, therefore, greater attention has been paid to fungi isolated from wood portion.

Botany Department Garden.—Five twigs were employed, all belonging to the same tree. The description of the twigs indicating the extent to which disease has progressed is given below :

Twig 1 (Text-fig. 1).—The upper part of the twig had been affected while the lower region remained green and apparently healthy. The affected part of the twig was slightly shrivelled and discoloured. There were no leaf-scars or wounds except a few superficial cracks of the bark. The leaves had wilted and turned brown. The twig was divided into five pieces.

Twig 2 (Text-fig. 2).—This twig showed a more advanced stage of the disease. The stem as well as the leaves had completely dried up. The stem showed a few leaf-scars, lenticels and a scar left by a broken branch. It was divided into seven pieces.

Twig 3 (Text-fig. 3).—This twig showed a very advanced stage of disease and the leaves had fallen off from one of the branches. There

were no leaf scars or wounds but lenticels were present. The upper end had a scar left by a fallen branch. It was divided into eight pieces. Four leaves were also tested.

Twig 4 (Text-fig. 13).—The whole twig had been diseased and it showed a very advanced stage. The twig was completely dry and shrivelled. Leaf scars, branch scars and lenticels were found on the stem. All the leaves had fallen off except for a few on two small branches and these leaves, too, were brown and dry. The twig was divided into 24 pieces.

Twig 5 (Text-fig. 5).—The entire twig had dried up and most of the leaves had broken off. Leaf scars and lenticels were present on the stem. The twig was divided into 10 pieces.

It will be seen from Text-figs. (1, 2, 3 and 13) that three out of the four twigs (Figs. 1–3) have produced *Botryodiplodia theobromæ* from wood from almost all the pieces. In twig 1 (Text-fig. 1) *Botryodiplodia theobromæ* has been found in the wood from the tip up to the base, excepting for one piece right at the end that gave only *Fusarium*. The two leaves tested from the top also gave rise to *Botryodiplodia theobromæ* only.

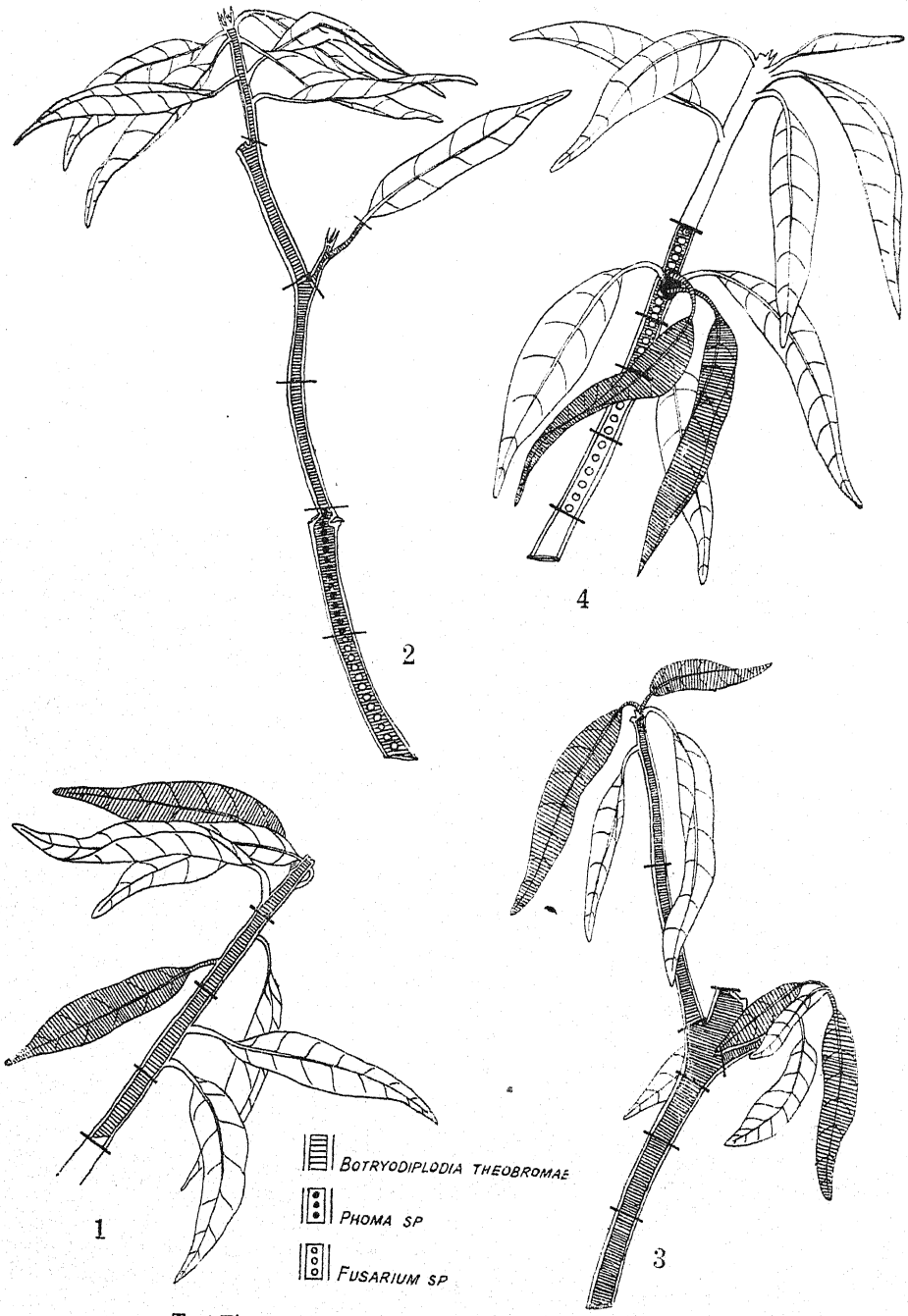
In twig 2 (Text-fig. 2) again *Botryodiplodia theobromæ* was present in the whole length of the diseased wood of both the branches tested, except two pieces at the end where mixed with *Botryodiplodia* there was *Phoma* in one and *Fusarium* in the other. The petiole of one leaf arising from the twig also gave *Botryodiplodia*.

In twig 3 (Text-fig. 3) *Botryodiplodia* was found all along the wood in all the three branches except at the dry shrivelled end of one branch from which all the leaves had fallen off. This piece gave rise to *Fusarium*. All the four leaves tested also gave rise to *Botryodiplodia*.

Twig 4 (Text-fig. 13), however, which was completely shrivelled gave entirely different result. *Phoma* was found in the wood of all the branches right up to the petiole of the leaves, which were still persisting but along with this fungus was found associated in certain places, *Fusarium*. Comparison with bark fungi showed that *Fusarium* in all these cases must have come from bark.

Twig 5 (Text-fig. 5) also showed practically the same result as twig 4. *Phoma* was present almost throughout the diseased twig, excepting *Fusarium* and *Alternaria* in restricted regions.

It is apparent from the result that in the first three twigs it is the *Botryodiplodia theobromæ* that causes the disease, the other associated fungi are secondary. In twigs 4 and 5 *Phoma* seems to be the casual fungus and *Fusarium* only secondary. The two diseases in these two sets of twigs should therefore be considered as different. This disease due to *Phoma* has only been found in dry shrivelled twigs at the last stage of the disease and no early stage of infection by *Phoma* has been found. It is still to be seen if these may represent the blight disease of mango twig described by Kanitkar and Uppal (1939).



Text-Figs. 1–4. Figures illustrating spatial distribution of the fungi in the diseased mango twigs

Figs. 1–3. Twigs from Botany Department. Fig. 4. Twig from Isabella Thoburn College.

Isabella Thoburn College Orchard.—A large number of twigs were collected of which only six twigs from one tree, and two from two others representing various stages of the disease were selected for investigation.

Tree 1, *Twig* 1 (Text-fig. 6).—The twig appeared healthy except for about two inches towards the lower end where it had turned black and slightly shrivelled. In this infected region there were leaf-scars and splits of the bark. The apparently healthy part of the twig was green and had a number of leaves and leaf-scars. The leaves though green were slightly pale and unhealthy looking.

The infected region was divided into two parts, the bark was removed from wood and each was put in a separate moist chamber.

Tree 1, *Twig* 2 (Text-fig. 4).—The only evidence of infection in this twig was slight blackening of the bark at the lower node where two of the leaves had wilted. A small globule of gum was secreted in between the petioles of these two leaves. All the other leaves remained green and apparently healthy. The lower part of the twig was divided into four pieces.

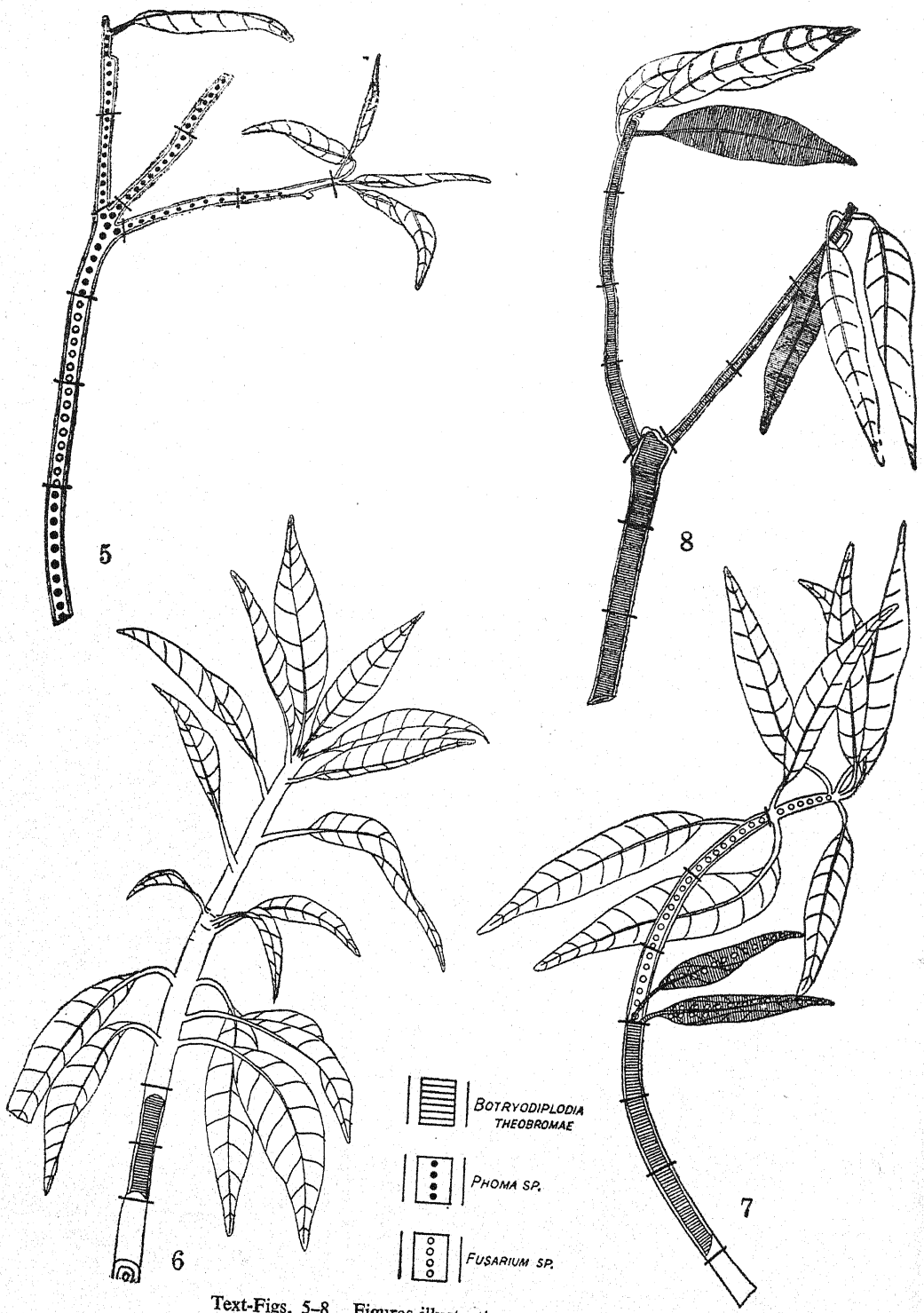
Tree 1, *Twig* 3 (Text-fig. 7).—This twig showed the initial stage of the disease. The infected region had turned black and two leaves at the upper end of the discoloured region had completely wilted. The part of the twig above the infected region had started wilting and the leaves were turning brown. The infected region was divided into 3 pieces and the apparently healthy part towards the apex into 4 pieces.

Tree 1, *Twig* 4 (Text-fig. 9).—A slightly advanced stage of the disease was shown in this twig. The upper end of the twig was discoloured and a brown colour extended along the midrib of the leaves of the infected region. The two edges of the leaves folded inwards and in some cases there was also a twisting of the leaves towards the leaf-scars, lenticels and splits in the bark. Globules of gum were found at different spots. The diseased portion of the twig was divided into six pieces and three of the leaves were also tested.

Tree 1, *Twig* 5 (Text-fig. 10).—The upper end of the twig was infected and the stem had turned brown. Leaf-scars, lenticels and a scar left by a fallen branch were present in the infected region of the twig. Gum was secreted a little below the branch scar. In the leaves, a brown colour extended along the midrib and margins which advanced inwards. The leaves rolled inwards and in some cases they had curled towards the tip. The infected region was divided into 8 pieces and three of the leaves were tested.

Tree 1, *Twig* 6 (Text-fig. 11).—This twig showed a more advanced stage. Leaf-scars and lenticels were present in the infected part and a big globule of gum was secreted towards the lower end of the infected region. The leaves were all brown and shrivelled. The twig was divided into 9 pieces.

Tree 2, *Twig* 1.—A more advanced stage of the disease was shown by this twig. The stem as well as all the leaves had completely dried and shrivelled, and one branch was devoid of leaves. The twig was divided into 18 pieces.



Text-Figs. 5–8. Figures illustrating spatial distribution of the fungi in the diseased mango twigs

Fig. 5. Twig from Botany Department. Figs. 6 and 7. Twigs from Isabella Thoburn College. Fig. 8. Twig from Madras.

Tree 3, *Twig* 1 (Text-fig. 12).—This twig showed a very advanced stage of the disease. The entire twig was completely dry and most of the leaves had broken off from the stem. It was divided into 15 pieces.

It will be seen from Text-figs. 4, 6, 7 and 9 to 12 that *Botryodiplodia theobromæ* is the most frequently occurring strain on eight different twigs belonging to three different trees. Twig. 1 (Text-fig. 6) shows the initial stage of infection and the infected region produces only *Botryodiplodia theobromæ*. The result from twig 2 (Text-fig. 4) is somewhat different although the twig is in early stage of infection. Two fungi are found associated together—*Botryodiplodia theobromæ* and *Fusarium*. *Botryodiplodia* is exclusively present in the leaves, and in the upper region of the infected part, but *Fusarium* extends to the lower portion of the diseased tissue where there is no *Botryodiplodia.*

In twig 3 (Text-fig. 7) *Botryodiplodia* is restricted to the lower region, *i.e.*, the diseased part of the twig. *Fusarium* on the other hand is restricted to the wood of the upper region which externally appeared to be free from disease. The leaves, however, show a mixture of both the fungi.

In twig 4 (Text-fig. 9) where the disease had advanced further the leaves and the entire length of the wood gave *Botryodiplodia*, only a small portion showed the presence of *Fusarium*.

In twig 5 (Text-fig. 10) the wood of the upper portion along with the leaves gave *Botryodiplodia*, lower half only *Fusarium*.

In twig 6 (Text-fig. 11) showing still more advanced stage of the disease, almost the entire length is *Botryodiplodia* which is mixed with *Fusarium* only at the lower end. There is pure *Fusarium* further down.

In twig 1 of tree 2 *Botryodiplodia* is present all along the wood except at the tip and in the small portion at the middle where *Fusarium* and *Phoma* are found.

In twig 1, tree 3 (Text-fig. 12) the entire wood of the infected part showed only *Botryodiplodia theobromæ*.

An analysis of the result shows that there is an overwhelmin evidence that the disease in all these twigs is caused by *Botryodiplodia*.

Madras twigs.—Twigs obtained from Madras were all dry and showed an advanced stage of the disease. Five twigs were tested. All of these gave identical results. Text-fig. 8 shows one of these twigs. It was divided into 10 pieces and the bark was separated from wood in all cases except in the topmost piece. Two of the leaves in two different branches of the twig were also tested. *Botryodiplodia theobromæ* was the only fungus obtained from the diseased bark, wood and leaf (Text-fig. 8) and undoubtedly the cause of disease.

Inoculation experiments

Mango plants.—Forty mango plants were raised from seeds and grown in pots for inoculation tests to find out whether *Botryodiplodia*, *Fusarium* and *Phoma* could parasitise vigorously growing healthy seedlings. Monohyphal agar cultures of the different strains were used in all the inoculation experiments. The plants were inoculated after

Text-Figs. 9–13. Figures illustrating spatial distritbution of the fungi in the diseased mango twigs Figs. 9–12. Twigs from Isabella Thoburn College. Fig. 13. Twig from Botany Department.

they had grown for five months. Most of the plants were about 1½ feet high while a few were only 1 foot in height.

The places to be wounded were first cleaned by swabbing with 0·1% mercuric chloride and then with sterile distilled water. An incision exposing the cells of the wood was made with a sterile scalpel and mycelium taken from the margins of agar cultures 3 days' old in *Botryodiplodia*, and 5 days' old in other 2 strains, was inserted in the wounds. Incised plants which received no inoculum served as controls. The wounded places were protected by wrapping with moist cotton and waxed paper. Of the 40 seedlings used, 10 replicates were made for each fungus—*Botryodiplodia*, *Phoma* and *Fusarium*, and the remaining 10 served as control.

None of the seedlings showed any sign of infection or wilting even though they were kept under observation for 6 months. A similar experiment with 40 seedlings next year also yielded negative results. Only *Botryodiplodia* was found to have produced in a few twigs slight canker near the inoculation point.

Mango twigs.—Botryodiplodia, *Phoma* and *Fusarium* were inoculated on healthy young twigs attached to the tree. Twenty-four twigs were selected for the purpose on the same tree. Six twigs were inoculated with each fungus and six served as control. Procedure adopted was the same as in inoculations on the young plants.

Out of six twigs inoculated with *Botryodiplodia*, three showed die-back symptoms on the 12th day after inoculation. All the twigs inoculated with the other strains remained unaffected. The three affected twigs on reisolation yielded only *Botryodiplodia*.

It will be seen from these inoculation experiments that all the four strains have failed to produce the disease on the inoculated young plants while *Botryodiplodia* is the only successful strain producing an infection of 50% on the inoculated twigs. Further inoculation experiments are in progress to find out the predisposing factors and the conditions under which the wounded parts get infected.

Discussion

The die-back of fruit and other trees is of common occurrence. Among stone fruit trees the disease is known to occur on almonds, apricots, peaches and plums (Cunningham, 1925), the causal organism, being *Clasterosporium carpophilum*. Die-back of apple branches due to *Glæosporium* sp. has been described by Wilkinson (1942).

The disease is also known to occur on Asiatic chestnuts which is caused by *Phomopsis* (Bedwell, 1937) and *Cryptodiaporthe castanea* (Tul.) Wehmeyer, *Botryosphæria ribis chromogena* G. and D. and *Diplodia* sp. (Fowler, 1938).

The white elm trees (*Ulmus americana* L.) in Nebraska is affected by die-back caused by *Cephalosporium* later identified as *Dothiorella ulmi* (May, 1931). The same disease in Elms in Minnesota is caused by a fungus which appears to be *Cytosporella*.

As regards Gymnosperms, Curtis (1926) reported a die-back of *Pinus muricata* and *Pinus radiata* by the fungus *Botryodiplodia piniea* in New Zealand. The die-back of fir (*Pseudotsuga Douglasii* Carr.) has been attributed to the attack of *Sphæropsis Ellisii* (Petri, 1913) and of *Pseudotsuga laxifolia* to that of *Diplodia pinea* (Waterman and Miller, 1936).

In India Petch (1916) has investigated the die-back of *Hevea brasiliensis* and die-back of tea plant of Ceylon which are ascribed to *Botryodiplodia theobromæ*. Sundararaman (1932) has reported die-back of cashewnuts by *Corticium salmonicolor*. This fungus according to him, is also known to attack mango, orange, jack-fruit, tea, coffee and several other trees. Narasimhan (1933-34) has reported *Diplodia* die-back of limes. Recently Kheswalla (1936) has reported die-back disease of fruit trees in Baluchistan by *Cytospora*. From a die-back of the tops of young Robusta coffee trees Mayne (1936) has isolated *Colletotrichum coffeanum*. Kanitkar and Uppal (1939) have given a short account of the twig blight of mango tree in Poona caused by species of *Phoma*.

In all these cases mentioned the disease is of fungal origin. But die-back may also be caused by bacteria, and by deficiency of salts.

The die-back of *Aucuba japonica* (Thunb.), for example, is caused by *Pseudomonas aucubicola* (Trapp, 1936). Ark and Thomas (1940) described the twig blight of apple tree in California in which he failed to find any pathogenic organism. On the other hand, the addition of boron and potassium in soil was found to reduce the die-back symptoms of the affected plants. Die-back of cloves in Zanzibar Protectorate is stated to be due to phosphorus and potassium deficiency owing to the deflection of these salts by grasses (*Ann. Rep. Dept. Agr. Zanz. Protectorate*, 1935, 1936).

Dwyer (1937) reports die-back deficiency disease in *Cocos nucifera* and also a physiological trouble affecting young palms which is characterised by a pronounced tip withering of the central leaves and drying-back of the outer leaves and pale-brown streaks on the back of the petioles.

Tubbs (1937) investigated factors affecting the die-back disease of tea, ascribed by Petch to *Botryodiplodia theobromæ* and is of opinion that the disease is of physiological origin and is associated with the deficiency in the production of carbohydrates ; tea plants producing only half the carbohydrate necessary for their growth.

A number of fungi has been found to be associated with the die-back of mango trees here investigated. Leaving aside those which are exclusively found on the bark and are definitely saprophytic, there are three fungi *Botryodiplodia*, *Phoma* and *Fusarium*, which have claim to be regarded as causal organisms. Association of a large number of fungi in a tree affected with die-back is not unknown. Bedwell (1937) found *Sphæropsis*, *Diplodia*, *Cytospora*, *Diplodina*, *Macrophoma Fusicoccum*, *Dothiorella*, *Phoma* and *Epicoccum*, associated with twig blight of Asiatic chestnut, along with the more important pathogen *Phomopsis*.

The reconstruction of the exact position of the fungi in the twig pieces showed that *Botryodiplodia* was exclusively present in the wood of a number of twigs suggesting that the disease is due to *Botryodiplodia*. The pathogenicity of *Botryodiplodia* has also been demonstrated by inoculation experiment in which the fungus was able to infect mango twigs through wounds although no vigorously growing plant showed such infection.

It appears that the disease of other two twigs of the Botany Department is caused by *Phoma*. Although the result of the preliminary inoculation experiment is negative, the fact that the fungus has been found to be permeating the wood of the entire diseased twig almost exclusively seems to indicate the pathogenicity of *Phoma*. Further inoculation experiment is necessary to settle the point.

Kanitkar and Uppal (1939) have also found a species of *Phoma* causing twig-blight of mango trees. It is evident from the spore size that the two species are different.

The die-back or twig blight of the mango trees is caused definitely by *Botryodiplodia* and probably also by *Phoma*. It is not rare to find more than one organism causing die-back of the same species of tree. White elm in different parts of America is a case to the point.

The pathogenicity of *Fusarium* is, however, doutbful. There is no diseased twig from which the fungus alone has been isolated. When associated with other fungi, *Botryodiplodia* or *Phoma* it has almost always been found in restricted regions, the other fungus predominating. In such cases *Fusarium* must be considered as a secondary organism.

The same *Fusarium* found in the twigs of Isabella Thoburn College is pathogenically more significant particularly in two cases.

In tree 1, twig 2 *Fusarium* is not only associated with *Botryodiplodia* at the diseased portion of the twig but also occupies the wood of the diseased portion at the base, where no *Botryodiplodia* has been found. Of more significance is the twig 3 of the same tree. Here *Botryodiplodia* is restricted to the diseased portion only, whereas *Fusarium* exclusively occupies the apparently healthy portion of the twig from above the diseased portion right up to the tip.

The presence of *Fusarium* in advance of *Botryodiplodia* in the apparently healthy tissue up to the tip may point to its being a pathogen, or it may only indicate, that once having penetrated in the wake of the actual pathogen, *Fusarium* can advance more quickly inside the tissue.

There is thus a gradation in the pathogenicity of *Botryodiplodia*, *Phoma* and *Fusarium*, the three fungi intimately associated with die-back. The two fungi *Botryodiplodia theobromæ* and *Phoma* have often been found to be separately associated with *Fusarium*. The possibility that in such cases the disease is due to the combined activity of the two fungi, although *Botryodiplodia* and *Phoma* may produce the disease independently cannot be completely overlooked.

Botryodiplodia has been known to cause die-back in various plants, for example, *Pinus*, rubber, tea, etc., and also associated with leaf-break

of palm leaf, ring disease of palm nuts (Dwyer, 1937), diseased pods of cocoa (Baker, 1936) and storage diseases of grape fruits (Wardlaw and Leonard, 1937). It is not definite, however, if in all these cases the disease is due to *Botryodiplodia*.

The production of the die-back may be occasioned by unfavourable conditions acting as predisposing factors. In such cases the adverse conditions affect the growth and vitality of the tree in question which easily succumb to the invasion of otherwise harmless facultative parasites.

Münch (1935) is inclined to believe that the die-back of Larches is due to a fungus but not the fungus *Dasysclypha Willkomii* commonly associated in Germany with Larch Canker. Grimm (1937), however, is of opinion that the cause of dying off of Larches in Germany was due primarily to the disturbances of transpiration associated with adverse environmental factors ; the trees thus weakened are readily accessible to infection by the Canker producing fungus. Day (1937) supports Langner's general conclusion (1936) that the fungus plays a secondary but a definite and necessary part in the development of the die-back and states that frost in this case is the predisposing factor.

Mayne (1935–1936) found that in the die-back of coffee tree the diseased shoots invariably showed the presence of *Colletotrichum coffeanum*, which was frequently the only fungus isolated. It was always found in shoots showing the very earliest external symptoms of the disease. But from field observations and the inconclusive inoculation experiments and other evidence he came to the conclusion that the primary predisposing factors in die-back are unfavourable conditions and premature leaf fall due to coffee-leaf-disease-fungus *Hemileia vestatrix*, and *Collectotrichum coffeanum* is only secondary to predisposing factors.

Müller (1936) describes that in the top die-back of coffee the presence of abundant shade of *Leucæna glauca* was observed to minimise the incidence of the disease. It is not improbable that high temperature in this case is the predisposing factor.

According to Dade (1937) swollen shoot of Cocoa is the result of extreme exposure of the tree to sun and wind during dry season, brought about by the gradual disappearance of shade trees and the surrounding forest (drought die-back). These weakened trees are invaded by secondary fungi such as *Botryodiplodia theobromæ* and saprophyte such as *Gliocladium roseum*, none of which can attack healthy tissue and produce necrotic die-back tissue with which these are always associated.

In the die-back of mango trees here investigated it seems that the fungi *Botryodiplodia* and *Phoma* are able to infect and produce the disease only in the less vigorous plants or twigs. It can well be that abnormal high summer temperature, 115° C., which sometimes kills twigs outright, is the predisposing factor.

The mode of infection and general symptoms of the disease are almost the same as described for other trees.

The infection in the "top die-back of coffee" caused by *Rhizoctonia* invariably commences in growing twigs and may frequently be detected before the hyphæ spread into stem. In the field the fungus travels from the leaves, through the wood vessels of the branches and stems. As a rule its diffusion is two to three times more rapid in an upward than in a downward direction (Müller, 1936).

In *Hevea brasiliensis*, the infection usually takes place not at the tip but at a variable distance from the growing point. The branch dies above the point of attack and less rapidly also backwards towards the base. As spread backwards occurs, the whorl of branches lower down are killed off in succession.

In the die-back of mango twigs caused by *Botryodiplodia* it has been found that the infection occurs at a node at variable distance below growing point, and the part of the twig above and below the point of infection dies. The leaves lose their healthy green colour and gradually turn brown. The browning starts at the base of the leaf and spreads along the midrib and then out along the veins to the margins. This is followed by the browning of the whole leaf, accompanied by the upward rolling of the margin.

In the white elm, the infection twigs show an internal streaking of the vascular tissues of the current season's growth. Goss and Frink (1934) have failed to find any external blackening of the bark above the point of infection although it has been described by May (1931) to occur on the Dutch Elm. In the infected mango twigs, the external blackening as well as internal streaking are clearly marked. The latter is seen as dark band between the xylem and cortex.

SUMMARY

The paper deals with the die-back disease of mango trees.

Botryodiplodia theobromæ, *Phoma* and *Fusarium* are the three fungi intimately associated with the die-back.

There is direct evidence from inoculation experiments that mango twigs can be infected and die-back produced by *Botryodiplodia*.

The evidence regarding the pathogenicity of *Phoma* is indirect, and based on the almost exclusive appearance of the fungus from some diseased twigs.

The pathogenicity of *Fusarium*, the other fungus associated with the disease, is not established.

These fungi are unable to attack vigorous healthy plants.

The predisposing factor in the case of die-back of Lucknow plants may be high summer temperature which affects the vitality of plants and enables the pathogens to attack.

The external symptoms and histopathology of die-back caused by *Botryodiplodia* have been given in detail.

LITERATURE CITED

1. Ark, P. A. and Thomas, H. E. (1940) "Apple die-back in California," *Phytopath.*, **30**, 148–54.

*2. *Annual Report of the Department of Agriculture, Zanzibar Protectorate*, 1935, 1936, 45 (Abs. *Rev. App. Myc.*, 1937, **16**, 301).

*3. Baker, R. E. D. (1936) "Notes on Trinidad fungi. I. Phytophthora," *Trop. Agriculture Trin.*, 13, 12, 330–32 (Abs. *Rev. App. Myc.*, 1937, 16, 312).

4. Bedwell, J. L. (1937) "Twig blight of Asiatic chestnuts, especially that caused by *Phomopsis*," *Phytopath.*, **27**, 1143–51.

5. Cunningham, G. H. (1925) *Fungous Diseases of Fruit Trees in New Zealand and their Remedial Treatment*, New Zealand Fruit Growers' Federation, Ltd., Brett Printing and Publishing Co., Auckland, 1–382.

*6. Curtis, K. M. (1926).. "A die-back of *Pinus radiata* and *P. muricata*, caused by the fungus *Botryodiplodia pinea* (Desm.) Petr., *New Zealand Inst. Trans. and Proc.*, **55**, 52–57.

*7. Dade, H. A. (1937) .. "Swollen shoot of Cacao," *Report on Mr. H. A. Dade's visit to the Gold Coast. Sess. Pap. Legist. Counc.*, Gold Coast Colony No. 5 of 1937, 15 (Abs. *Rev. App. Myc.*, 1938, **17**, 224).

8. Das Gupta, S. N. and Zachariah, A. T. (1939) "On the 'die-back' disease of mango tree," *Proc. 26th Ind. Sci. Congress, Lahore*, 117.

*9. Day, W. R. (1937) .. "The dying of Larch: a note on Professor E. Münch's monograph "Das Lärchensterben." *Forestry*, **11**, No. 2, 109–16 (Abs. *Rev. App. Myc.*, 1938, **17**, 360).

*10. Dwyer, R. E. P. (1937) "The disease of coconuts (*Cocos nucifera*) in New Guinea," *New Guinea Agri. Gaz.*, **3**, 1, 28–93 (Abs. *Rev. App. Myc.*, 1937, **16**, 669–70).

*11. Ferrer, B. B. (1936) .. "El problema de los cultivos de Arroz y cacao en el cauca," *Agricultura, Bogata*, **8**, 2, 90–91 (Abs. *Rev. App. Myc.*, 1937, **16**, 202).

12. Fowler, M. E. (1938) "Twig cankers of Asiatic chestnuts in the Eastern United States," *Phytopath.*, **28**, 693–704.

13. Goss, R. W. and Frink, P. R. (1934) "*Cephalosporium* wilt and die-back of the white elm," *Univ. Nebraska Agr. Exp. Sta. Research Bull.*, **70**.

*14. Grimm, W. (1937) .. "Beitrag zur Losung des Larchenratsels," *Forstwiss. Zbl.*, **59**, 16, 501–12 (Abs. *Rev. App. Myc.*, 1938, **17**, 214).

15. Kanitkar, U. K. and Uppal, B. N. (1939) "Twig blight and fruit rot of Mango," *Curr. Sci.*, **8**, 470–71.

*16. Kheswalla, K. F. (1936) "Fruit diseases in Baluchistan," *Agri. Live-Stk. India*, **6**, 204–15 (Abs. *Rev. App. Myc.*, 1936, **15**, 586).

*17. Langner, W. (1936) .. "Untersuchungen über Lärchen-, Apfel- und Buchenkrebs," *Phytopath. Zeit.*, **9**, 111–45 (Abs. *Rev. App. Myc.*, 1936, **15**, 693).

*18. May, C. (1931) .. "A new elm disease," *Science* (N.S.), **74**, 437.

*19.	Mayne, W. W. (1935)	"Annual Report of the Coffee Scientific Officer, 1934–35," *Bull. Mysore Coffee Exp. Sta.*, **13**, 28. (Abs. *Rev. App. Myc.*, 1936, **15**, 16–17.)
*20.	———— (1936)	"Annual Report of the Coffee Scientific Officer, 1935–36," *ibid.*, **14**, 21. (Abs. *Rev. App. Myc*, 1936, **15**, 798.)
*21.	Muller, H. R. A. (1936)	"Topsterfte van Koffie," *Arch. Koffiecult Ned-Ind.*, **10**, 280–349. (Abs. *Rev. App. Myc.*, 1937, **16**, 670–72.)
*22.	Munch, E. (1935) ..	'Das Lärchenrätsel als Rassenfrage. Zweite Mitteilung: Die Lärche in Seeklima," *Z. Forst-U. Jajdw.*, **67**, No 8, 421–42; No. 9, 483–500. (Abs. *Rev. App. Myc.*, 1936, **15**, 131.)
23.	Narasimhan, M. J. (1935)	"Report of the mycological section for the year 1933–34," *Admin. Rep. Agri. Dept. Mysore.* 1933–34, 19–22. (Abs. *Rev. App. Myc.*, 1936, **15**, 77.)
*24.	Petch, T. (1916) ..	"Diseases of *Hevea* in Ceylon," *Trans. 3rd International Congress of Tropical Agriculture*, **1**, 596–607.
*25.	Petri, L. (1913) ..	"Disseccamento die rametti di *Pseudotsuga Douglasii* Carr. prodotto da una varietà di *Sphæropsis ellisii* Sacc.," *Ann. Mycol.* **11**, 278–880.
26.	Sundararaman, S. (1932)	"Die-back disease of cashewnuts—West coast," *Department of Agriculture, Madras, Leaflet*, No. 46.
27.	Trapp, G. (1936) ..	"A bacillus isolated from diseased plants of *Aucuba japonica* (Thunb.)," *Phytopath.*, **26**, 257–65.
*28.	Tubbs, F. R. (1937) ..	"On the growth and carbohydrate supply of the Tea plant after pruning." *J. Pomol.*, **14**, 4, 317–46. (Abs. *Rev. App. Myc.*, 1937, **16**, 494–95.)
*29	Wardlaw, C. W. and Leonard, E. R. (1937)	"Antiseptic and other treatments in the storage of Trinidad Citrus fruits," *Mem. Low. Temp. Res. Sta. Trin.*, **5**, 3–23. (Abs. *Rev. App. Myc.*, 1937, **16**, 559–60.)
30.	Waterman, A. M. and Miller, J. A. (1936)	'A die-back of Douglas fir," *Phytopath.*, **26**, 804–05.
*31.	Wilkinson, E. H. (1942)	"Die-back and canker of apple branches caused by a *Gloeosporium* sp. Gdnrs.," *Chron.*, Ser, 3, **111**, 2896, 269. (Abs. *Rev. App. Myc.*, 1942, **21**, 419.)

* Literature not consulted in original.

EXPLANATION OF PLATE V

Figs. 1–2. Illustrating general aspect of a mango tree from Madras affected with die-back.

1. A diseased tree showing dead shrivelled twigs bare of leaves among green foliage.
2. A close-up view of the diseased tree in Fig. 1.

Figs. 3–5. Illustrating external symptoms of early stage of die-back.

3. (*a*) Discolouration and shrivelling of the infected region of stem. (*b*) Leaves above the point of infection withering.
4. (*a*) Shrivelled young twig ; (*b*) upper three leaves have wilted .
5. (*a*) Infection at a node where all the leaves have wilted ; (*b*) the leaves at the top apparently healthy.

Figs. 6–7. Illustrating external symptoms of advanced stage of die-back.

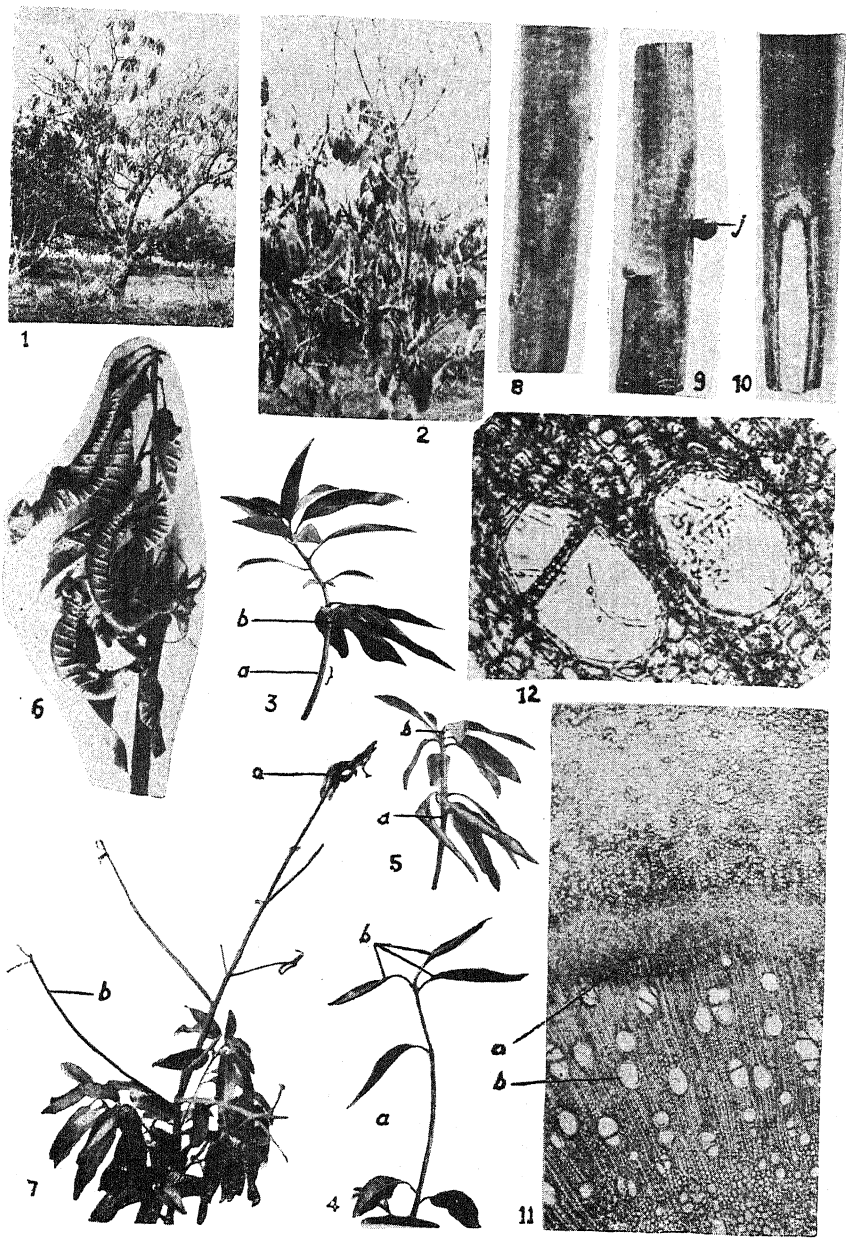
6. Characteristically curled leaves on the shrivelled twig enlarged.
7. Final stage of dead twigs. (*a*) Bunches of dry, curled leaves at the top ; (*b*) twigs devoid of leaves.

Figs. 8–10. Illustrating external symptoms at the early stage of infection.

8. Stem showing discolouration of the bark at the infected region—dark against green stem. ×1·5.
9. Side view of Fig. 1 showing the discoloured infected region and the gum globule (*g*). ×1·5.
10. Twig cut slantingly exposing the wood and internal streaking in phloem (*p*) and cambium (*c*). ×1·5.

Figs. 11–12. Histopathology : advanced stage.

11. Section of old infected region. ×52. (*a*) Brown deposits in phloem and cambium ; (*b*) xylem vessels with fungal hyphæ.
12. Xylem vessels (of Fig. 11) enlarged to show the hyphæ inside. ×330.



S. N. DAS GUPTA AND A. T. ZACHARIAH (MISS)—
STUDIES IN THE DISEASES OF MANGIFERA INDICA *LINN.*

STUDIES IN THE DISEASES OF *MANGIFERA INDICA* LINN.

V. The Structure and Development of Lenticels in the Mango Fruits

BY G. S. SINHA

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INTRODUCTION

MANGOES have recently received much attention in India and various diseases of the mango fruit are being investigated. The role of fruit lenticels as passages of infecting organisms has been emphasized by several workers (Kidd and Beaumont, 1925; Baker and Heald, 1932 and others) from time to time. The 'black-tip' disease of mango fruit recently reported (Das Gupta and Verma, 1939) seems to be frequent in orchards situated near brick kilns. If the gases emanating from the kilns have any direct effect on the fruits, the role of lenticels as channels of gaseous exchange may not be insignificant. It is for these reasons that the present investigation has been carried out.

The term 'lenticel' was originally adopted by De Candolle for such structures occurring on stems of flowering plants. In structural details the lenticels of fruits and stems differ. The layer of cambium cells invariably present in lenticels of the stem is usually lacking in the mango and other fruits. Nevertheless the same term may be applied in the fruits as a matter of convenience. Clements (1935) has used the term 'lenticel' in the case of pomes of *Pyrus Malus* and has rightly pointed out that the use by some workers of the names like fruit 'spots', 'dots', or 'pits' to denote lenticels of fruits involves a certain amount of confusion. These terms are liable to be misunderstood since the same expressions have often been employed by plant pathologists to indicate symptoms of certain fruit diseases. The pome being morphologically a stem structure the use of the term 'lenticel', in the author's opinion, is still more justified. In the case of true fruits such as mangoes, the essential structure of the lenticels is the same as in pomes of *Pyrus Malus* and therefore in the present paper the term 'lenticel' has been retained and refers to the small spots seen on the skin of healthy mango fruits. Lenticels without the characteristic cambium, designated as 'ventilating pits', have been reported in petioles of members of the Cyatheaceæ and the Marattiaceæ by Haning (ex. Haberlandt, 1914), but for reasons stated above Haning's terminology need not be followed for fruits.

MATERIAL AND METHODS

Three varieties of mango fruits, namely *safeda*, *dasehri* and *bambai*, were selected for study. Fruits in various stages of development starting from the youngest stage to the very mature one were collected from

Begum Bagh and Sikandarbagh orchards, Lucknow, during the months of March, April and May 1941. Epidermal peelings of these fruits were made in order to study the stomatal and lenticel structures in surface view. The peelings were made by treating small pieces of fruits with strong nitric acid and potassium chlorate for 12–24 hours, depending upon the stage of maturity of fruits. The soft part including the entire mesocarp was thus macerated and by shaking the treated pieces in water in a test-tube the epidermal peelings separated neatly from the rest of the tissue. The peelings were then treated with strong ammonia solution, washed in water and mounted in glycerine. Pieces of fruits were also embedded in paraffin and microtomed to help in the study of the structure of mature and developing, as well as open and closed lenticels. The open and closed lenticels were ascertained by the technique followed by Clements (1935) for *Pyrus Malus*. The fresh fruits were dipped in a solution of methyl blue at room temperature (30° C.) and later transferred to a cold chamber at 15° C. for about 24 hours. In so doing the skin of the fruit contracts and the lenticels are subjected to a mild expansion without being ruptured. The coloured solution passes through the open lenticels which show a halo of the dye while the closed lenticels remain uncoloured.

The number of lenticels, closed and open, was ascertained in all the three varieties of fruits by direct counts.

Structure of Lenticels

The lenticels appear as minute specks on the skin of fruits. The spots are of different sizes in the three varieties of mango. They are bigger in *bambai* than in *safeda* and *dasehri*, the last having the smallest spots among the three. The apparent colour of the lenticels ranges from light to dark brown and is often brick red as in *safeda*.

In surface view, as seen in peelings of the epidermis of mature fruits under the microscope, the lenticel region shows an opening of an irregular form surrounded by much divided subsidiary (of stomatal origin) and epidermal cells which radiate from the opening in all directions (Pl. VI, Figs. 1, 2, 3).

In vertical sections the lenticel apertures are seen as breaks in the epidermis (Text-figs. 1, 2 and 3). Below the opening a number of hypodermal cells are loosely packed, the number depending upon the size of the lenticels. These hypodermal cells are filled with a tannin-like substance to which the apparent colour of the lenticels is due. In fruits having larger lenticels the hypodermal cells seem to radiate from the opening. This is due to the pulling force acting on the hypodermal cells during the stretching of the epidermis as the fruit matures and grows in size. Below the hypodermal cells are ordinary parenchymatous cells of the pericarp. Unlike lenticels of stems of angiosperms, the lenticels in mango fruits do not show any sign of development of a cambium under the loosely packed hypodermal cells, and in this respect differ from them. As already stated, it is the absence of this cambium that should evoke difference of opinion as to the validity and correctness of the use of the term 'lenticel' (primarily employed to describe the lenticels of stems of angiosperms) for such structures on fruits.

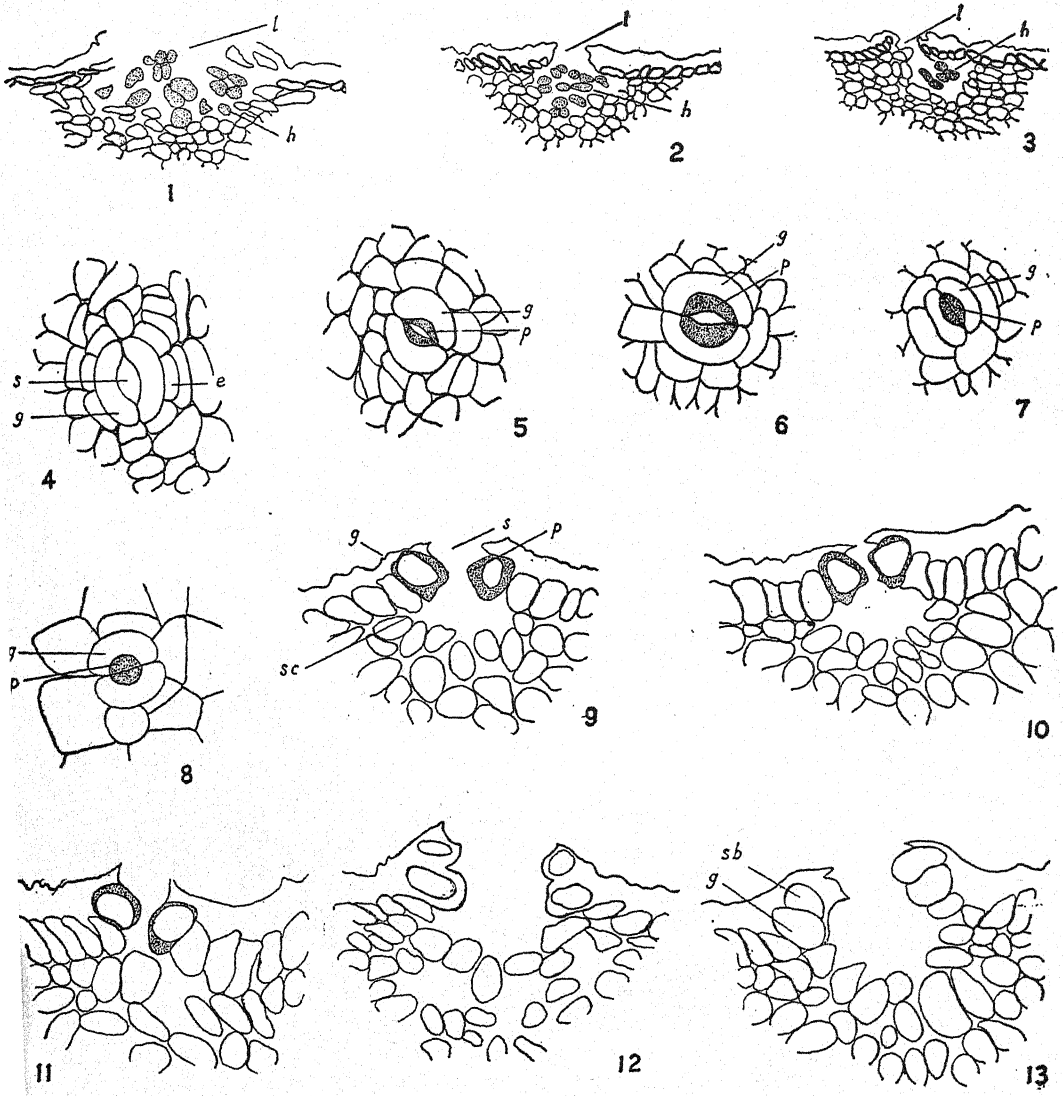
Development of Lenticels

Lenticels which occur invariably on the skin of mature mango fruits are, however, not percievable when the fruits are very young. An examination of the epidermal peelings of fruits of this age reveals that the skin bears a number of stomata, but no lenticels are found.

The stomatal apparatus consists of two large guard cells with a substomatal chamber below and a stomatal pore above (Text-figs. 4–11). The guard cells have their walls thin on the side away from the pore while round the latter the wall is very thick, forming a thickened poral rim. The poral rim is much more clear in surface view when the stomatal pore is closed than when it is open. In the region of the poral rim the guard cells have projecting cuticular ridges which in a section appear as horn-like cuticular projections from the guard cells and partly overarch the stomatal pore (Text-figs. 9–11).

Subsidiary cells which usually accompany guard cells in many angiosperms seem to develop, in the case of mango fruits, after the guard cells are fully distinct. In very young stages of the fruit the guard cells are surrounded by ordinary epidermal cells (Text-figs. 4–8), but later on an enlarged cell appears on the lateral side of each guard cell. Thus on the side of each guard cell appears a lateral subsidiary cell. No polar subsidiary cells have been found to develop in this case. As the fruits grow the lateral subsidiary cells begin to overarch the guard cells (Text-figs. 14–17). Each subsidiary cell then divides transversely into three or sometimes four cells, which further overarch the guard cells (Text-figs. 18–20, and Pl. VI, Figs. 4–6). This type of subsidiary cells has also been reported in some angiosperms (Bandulska, 1924, 1926) and the Benettitales (Florin, 1933). In vertical sections of the stomata at this stage the guard cells are seen to be superposed by the subsidiary cells (Text-figs. 12, 13).

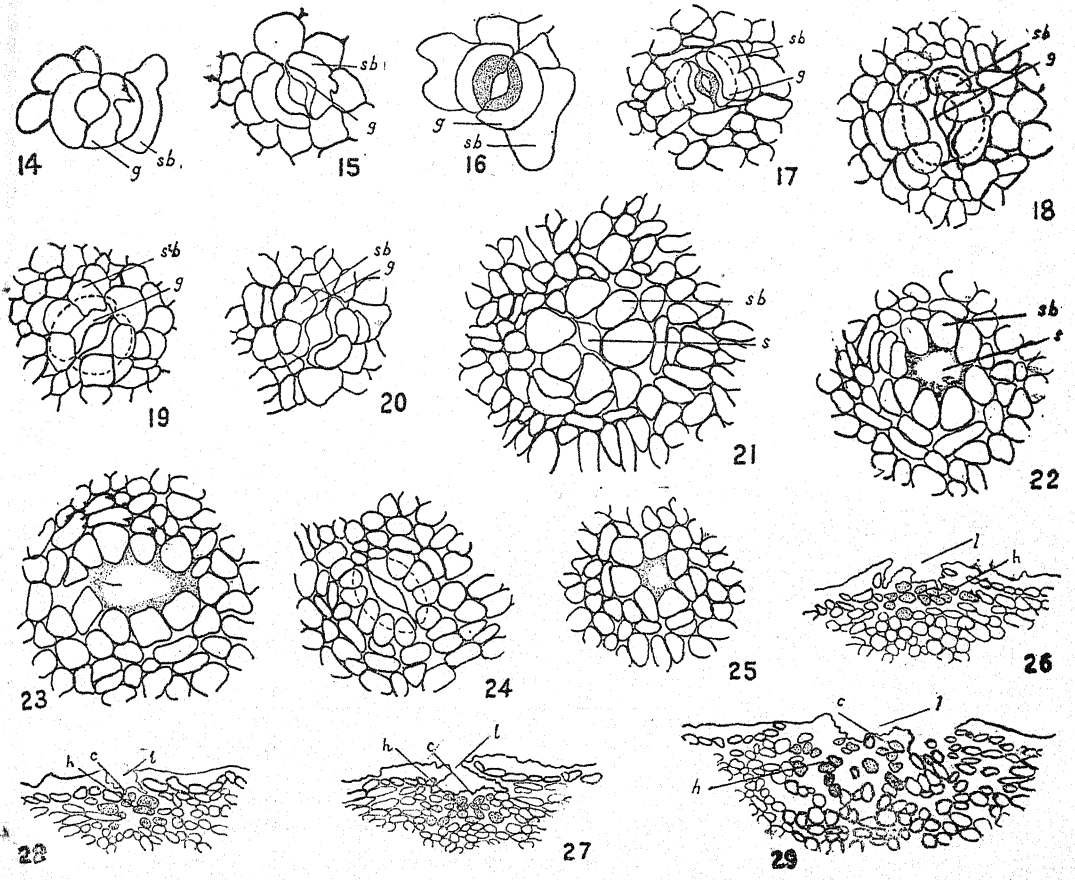
The first step in the development of lenticels is the permanent opening of the guard cells. The lateral subsidiary cells further divide and completely overarch the guard cells leaving only the stomatal pore. In surface view these cells lie round the pore (Text-figs. 21–25). By further division the subsidiary cells merge into the adjoining epidermal cells and in so doing they spread radially from the stomatal pore, probably under the force caused by the stretching of the epidermis as the fruits mature (Pl. VI, Figs. 1–3). Due to the stretching of the epidermis on all sides, the pore is widened and forms the lenticel aperture. At the same time the hypodermal cells below the substomatal chamber undergo change, under the same influence, which creates intercellular spaces among them (Text-figs. 1–3). The hypodermal cells get a deposit of tannin-like substance to which the colour of the lenticels, in surface view, is due. The number of hypodermal cells taking part depends on the size of the lenticels. Thus the least number is involved in *dasehri*, where the lenticels are the smallest, and the largest in *bambai* where the lenticels are the biggest among the three varieties studied. During these changes the guard cells of the stomata remain intact for quite a long time and can be recognised particularly in surface view. The shape and form of the guard cells do not, however, remain the same



Text-Figs. 1–13.—Figs. 1–3. Lenticels in vertical sections. *l*, lenticel aperture ; *h*, loosely packed hypodermal cells. 1. *bambai;* 2. *safeda* ; 3. *dasehri.* ×94. Figs. 4–8. Stomata in surface view. *s*, stomatal pore ; *g*, guard cells ; *e*, epidermal cell ; *p*, poral rim. 4–5. *bambai;* 6–7. *safeda;* 8. *dasehri.* ×375. Figs. 9–11. Same in vertical sections. *sc*, substomatal chamber. 9. *bambai;* 10. *safeda;* 11. *dasehri.* ×375. Figs. 12–13. Same when guard cells are overarched by subsidiary cells (*sb*). 12. *bambai;* 13. *safeda.* ×375.

when seen in a vertical section. It is to be pointed out here that all the stomata in a fruit do not develop into lenticels. In such cases the

guard cells shrivel and the pore becomes permanently closed and as the fruit enlarges they can be seen lying somewhat disorganised among the developing lenticels.



Text-Figs. 14–29.—Figs. 14–25. Stages in development of lenticels from stomata, as seen in surface view. *s*, stomatal pore; *g*, guard cell; *sb*, subsidiary cell. 14, 21, 22. *bambai.* ×375. 17, 18. *bambai.* ×240. 15, 19. *safeda.* ×240. 23. *safeda.* ×375. 16. *dasehri.* ×375. 20, 24, 25. *dasehri.* ×240. Figs. 26–28. Vertical sections of closed lenticels. *l*, lenticel aperture; *h*, hypodermal cells; *c*, cuticle. 26. *bambai;* 27. *safeda;* 28. *dasehri.* ×84. Fig. 29. *bambai.* A partially closed lenticel in vertical section. ×84.

As the fruits grow some of the lenticels become closed, while the rest remain open. Closed and open lenticels were ascertained by the technique followed by Clements (1935) for apples and described above. The closing of the lenticels has been found to occur by the development of a layer of cuticle on the outermost layer of the hypodermal cells. The extent of development of this layer of cuticle determines

the complete or partial closure of the lenticels. In fully closed lenticels, the cuticle develops over the whole length of the hypodermal cells (Text-figs. 26–28), while in partly closed ones the cuticle does not cover some of the hypodermal cells (Text-fig. 29). Lenticels, in which the cuticle does not at all cover the hypodermal cells, are regarded as open (Text-figs. 1–3).

Distribution of Lenticels in Mature Fruits

It was thought desirable to note the distribution of lenticels, closed and open, on the surface of the fruits. For this purpose a piece of graph paper divided in square centimeters was spread over the surface of the fruit and by pricking a needle through the paper, the four corners of the squares were marked off on the skin of the fruit. These squares were then completed on the skin of the fruit with ink and direct counts of lenticels in these areas were made under a magnifying lens. Counts were made for the total number of lenticels, both open and closed, per unit area in the basal and apical halves of the fruit, and also for open and closed lenticels separately, in any region of the fruit. The unit area taken was 9 sq. cms. in order to cover sufficient surface. Twelve fruits of each variety were taken and in each fruit different regions of the halves were covered in counting the number of lenticels.

Table showing the number and distribution of lenticels per 9 sq. cms. in mature fruits

Variety of Fruit	Total number of lenticels both open and closed		Number of open lenticels	Number of closed lenticels
	Basal half	Apical half		
safeda ..	190–198	240–248	25–36	160–172
dasehri ..	190–196	260–266	35–40	150–215
bambai ..	70–79	100–120	65–81	7–11

It will be noticed from the above table that the total number of lenticels in *bambai* is the least, while there is no marked difference between *safeda* and *dasehri*. It is also evident that lenticels are more numerous in the apical half of the fruit than in the basal half. In *safeda* and *dasehri* more of the lenticels are closed than open, while the reverse is the case in *bambai*.

Conclusion

It is evident from the foregoing observations that the skin of the mature mango fruit has numerous lenticels which look like tiny spots to the naked eye. In structure, there is a general correspondence with the lenticels found on stems of angiosperms, although there are differences in details. In the first instance, the fruit lenticels are comparatively very small in size, the number of hypodermal cells

involved being just a few. The hypodermal cells do not characteristically radiate from the lenticel opening in all cases as is the case with stem lenticels. Another important difference is the absence of the cambium cells below the hypodermal cells in fruit lenticels, while in lenticels of stem the cambium is conspicuously developed (Haberlandt, 1914). It is the absence of this cambium that has led to the discussion on the correctness or otherwise of the use of the term 'lenticel' in the case of fruits.

It has been found that the lenticels in mango fruit develop from stomata as also observed by several other workers (Zschokke, 1897, Tetley, 1930, Clements, 1935) for apples. In the development of the lenticel from the stoma it has been observed that the lateral subsidiary cells of the guard cells play an important role. The subsidiary cells, having divided transversely into several cells, gradually overarch the guard cells leaving only the stomatal pore which is now permanently open. It is the stomatal pore that forms the lenticel aperture which widens up rather irregularly under the stretching force of the enlarging fruit. Under the same influence the subsidiary cells along with the epidermal cells radiate from the pore in all directions, giving the lenticel region a characterstic appearance in surface view. At the same time some of the hypodermal cells below the substomatal chamber get loosely arranged and develop deposits of tannin which gives the brown colour to the lenticel. The overarching of guard cells by subsidiary cells has been observed before in leaves of angiosperms (Bandulska, 1924, 1926) and the Benettitales (Florin, 1933), but has not been reported for fruits so far in author's knowledge. In mature fruits some of the lenticels become closed by the development of cuticle on the outermost layer of hypodermal cells, while others remain open.

A study of the number and distribution of lenticels shows that certain varieties (*safeda* and *dasehri*) have more lenticels per unit area than others (*bambai*), and also that they are more in the apical half of the fruit than in the basal half. As regards the open and the closed lenticels it has been found, that in *safeda* and *dasehri* more of the lenticels are closed than open, while reverse is the case in *bambai*.

SUMMARY

The structure and development of lenticels in mango fruits have been described. The lenticels have been found to develop from stomata which alone are present in young fruits. The stomata become permanently open and the guard cells are gradually overarched by lateral subsidiary cells which divide and merge into the cells of the epidermis. As a result of the stretching, to which the epidermal cells of the enlarging fruit are subjected, the stomatal pore also enlarges, forming the lenticel aperture, from which the subsidiary and the epidermal cells seem to radiate. The hypodermal cells below the stomatal pore also come under the influence of stretching and become loosely arranged in rows radiating from the pore. Their walls become brown. The cambium, a usual feature below the hypodermal cells in stem lenticels, is absent in the lenticels of mango fruits. In the

mature fruit some of the lenticels become fully or partly closed by a complete or partial development of a layer of cuticle over the hypodermal cells.

All the stomata do not develop into lenticels. Lenticels are more numerous in the apical half of the fruit than in the basal half.

ACKNOWLEDGMENT

I wish to express my deep gratitude to Dr. S. N. Das Gupta for his help and guidance during the course of this investigation and to Prof. B. Sahni for permitting the use of his library.

LITERATURE CITED

Baker, K. F., and Heald, F. D. (1932) "The importance of lenticel infection of apples by *Penicillium expansum*," *State Coll. Washington Agr. Exp. Sta. Bull.*, 264.

Bandulska, H. (1924) .. "On the cuticles of recent and fossil Fagaceæ," *Jour. Linn. Soc. Bot.*, **46**, 427.

———— (1926) .. "On the cuticles of some fossil and recent Lauraceæ," *ibid.*, **47**, 383.

Clements. H. F. (1935) .. "Morphology and physiology of the pome lenticels of *Pyrus Malus*," *Bot. Gaz.*, **97**, 101.

Das Gupta, S. N. and Verma, G. S. (1939) "Studies in the diseases of *Mangifera indica* Linn. I. Preliminary observations on the necrosis of mango fruit with special reference to external symptoms of the disease," *Proc. Ind. Acad. Sci.*, **9 B**, 13.

Florin, R. (1933) .. "Studien über die Cycadales des Mesozoicums nebst erörterungen uber die spaltöffnungsapparate der Bennettitales," *K. Suenska Vet. Handl.*, Stockholm, Ser. 3, Band 12, No. 5.

Haberlandt, G. (1914) .. *Physiological Plant Anatomy*.

Kidd, M. N., and Beaumont, A. (1925) "An experimental study of the fungal invasion of apples in storage with particular reference to invasion through lenticels," *Ann. App. Biol.*, **12**, 14.

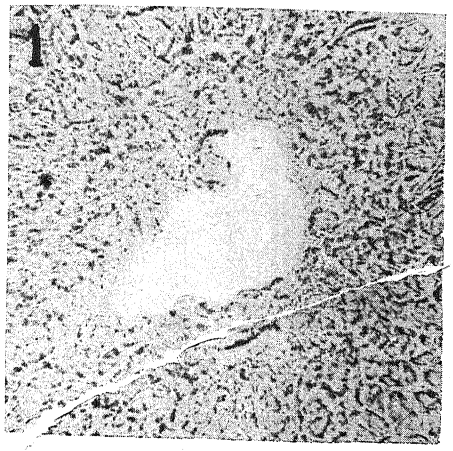
Tatley, U. (1930) .. "A study of anatomical development of the apple and some observations on the pectic constituents of the cell wall," *Jour. Pomology and Hort. Sci.*, 8, 153 (original not seen).

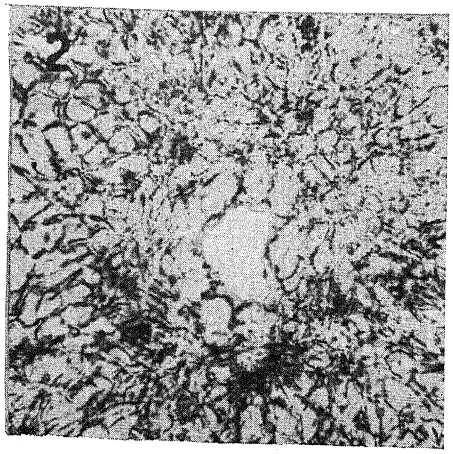
Zschokke, A. (1897) .. "Uber den Bau der Haut und die Ursachen der Verschieldenen Halt barkeit unserer Kernobot-früchte," *Landw. Jarhesb. Schweiz.*, 11, 196 (original not seen).

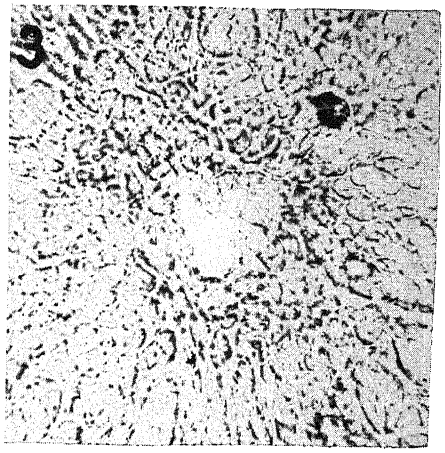
EXPLANATION OF PLATE VI

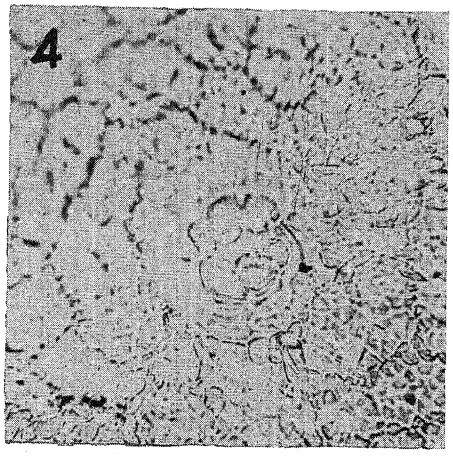
Figs. 1–3. Lenticels in surface view. (1) *bambai*, (2) *safeda*, (3) *dasehri*. All ×200.

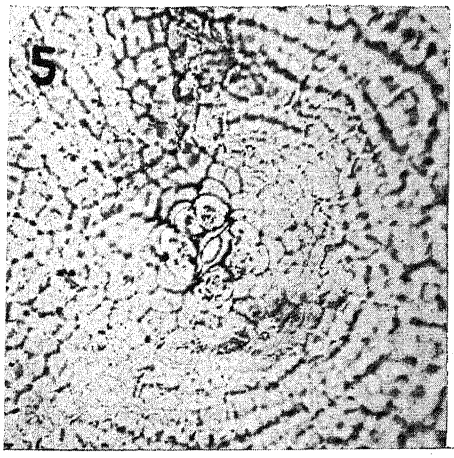
Figs. 4–6. Surface view of stomata overarched by the lateral subsidiary cells. (4) *bambai*, (5) *safeda*, (6) *dasehri*. All ×200.

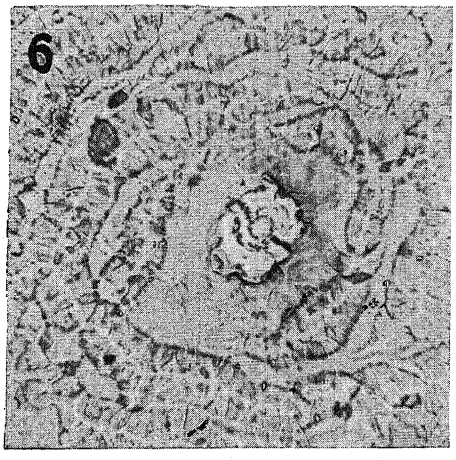




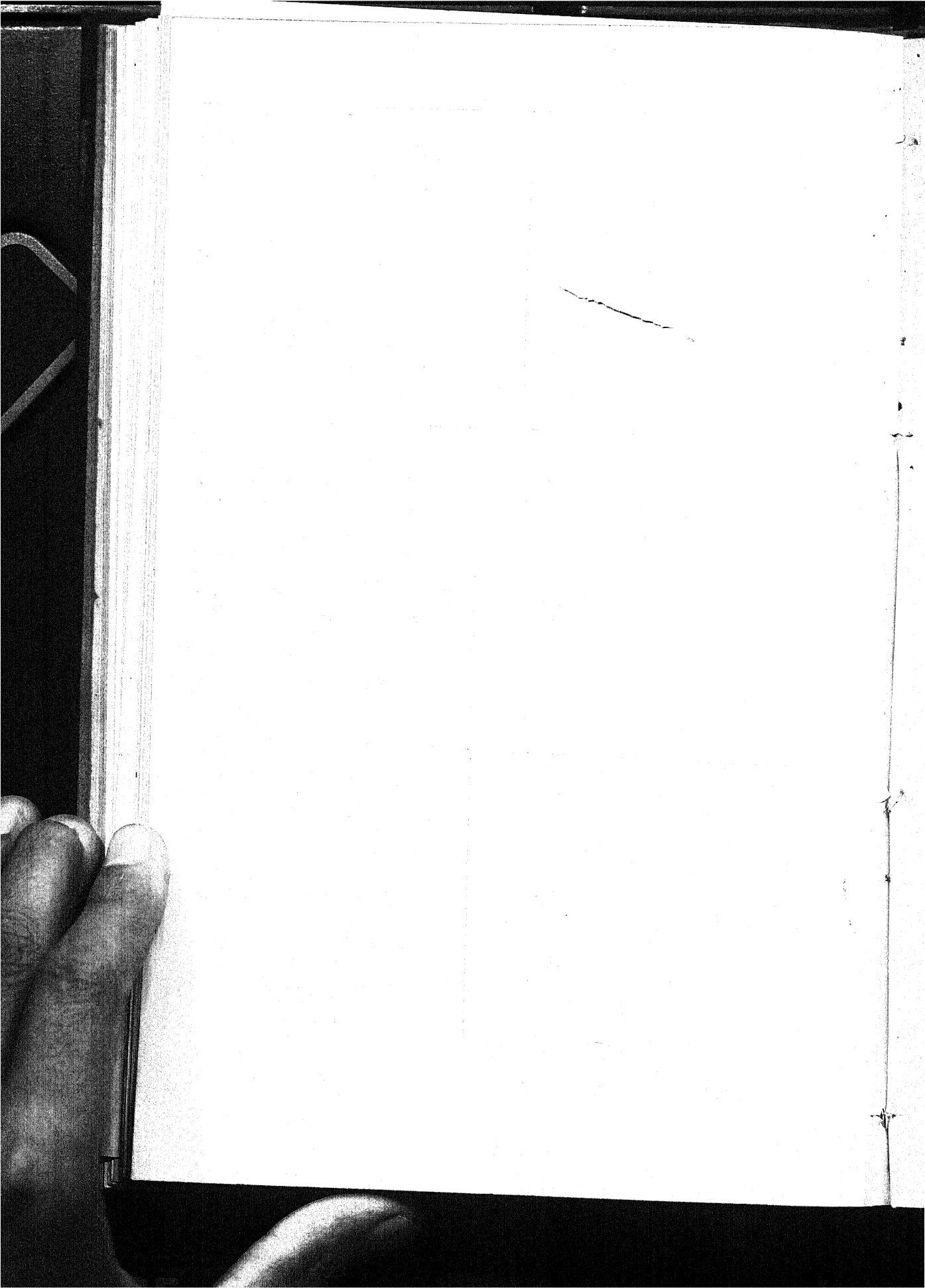








S. SINHA—*STRUCTURE AND DEVELOPMENT OF LENTICELS IN—*



A REVISION OF THE INDO-BURMESE SPECIES OF *LINDERNIA* ALLIONI

By S. K. Mukerjee

Llyod Botanic Garden, Darjeeling

Received for publication on February 14, 1945

The species properly referable to this genus have received different treatments from different taxonomists and have been placed under 2, 3 or even 4 separate genera, *viz.*, *Lindernia*, *Vandellia* Brown ex Linn., *Ilysanthes* Raf. and *Bonnaya* Link and Otto. The authors in most cases differ in their opinion in placing the species under these genera and many of them, realising that the line of demarcation is too fine between these artificial genera, have freely confessed their difficulties in trying to keep them separate. The character, on which the taxonomists who are in favour of keeping the four genera separate mostly depend, is the number of fertile stamens, 4 in some cases, and 2 in others with 2 staminodes. These staminodes show transitional stages and vary in different specimens of the same species. Cases are recorded where species which should have all the stamens fertile, have in some specimens two barren stamens.

Lindernia and *Vandellia*, both having 4 fertile stamens, are treated as separate by Linnæaus, Urban and few others, but have been combined by Bentham* and many others, and the generic name used, was *Vandellia* Linn., *Lindernia* All., or *Pyxidarsia* Hall. *Ilysanthes* and *Bonnaya* have 2 fertile stamens and 2 staminodes. In *Ilysanthes*, the fertile stamens are posterior and the staminodes anterior, while in *Bonnaya* the arrangement is just the reverse. For this reason, these two have been treated as distinct by Bentham, J. D. Hooker, Urban and a few others but have been united by Wettstein, Otto Kuntze, Hemsley and Skan, Hutchinson and Dalzell and most of the subsequent authors.

Thus we find that most of the taxonomists agree that there should be 2 genera instead of 4. Haines in his *Botany of Bihar and Orissa* (pp. 630–635), has also reduced the 4 genera into 2 but in a different manner. He has united *Bonnaya* with *Vandellia* and *Ilysanthes* with *Lindernia*. In reducing *Bonnaya* to *Vandellia* he makes the following remarks :—*"The genus *Bonnaya* differs from *Vandellia* as defined in the *Genera Plantarum* of Bentham and Hooker only by two of the stamens not bearing fertile anthers. But in view of the following considerations the separation of these two genera on this character alone appears

* Bentham at first treated *Vandellia* as distinct from *Lindernia* in DC. Prod. X, 418, but later combined the two under *Vandellia* in his *Scroph. Ind.* and in *Genera Plantarum.*

to me artificial and untenable. In *V. molluginoides*, Hooker found in some specimens 2 filiform staminodes hooked near the top, and I have a specimen of *V. Crustacea* in which the two anterior filaments appear to be without anthers. Some species of *Vandellia* can only be separated from species of *Bonnaya* by this one sexual character, *e.g.*, forms of *V. angustifolia* and *V. verbenæfolia*. Finally the character of the barren stamens themselves differs and shows transitional stages. In *Bonnaya verbenalfolia*, the anterior stamens in some specimens bear anthers but these are small and unfertile. In *B. veronicæjolia* there are no anthers, but the filaments are long and curved or hooked as in the case of some *Vandellia molluginodes*. In *Bonnaya brachiata* the barren stamens are short pubescent scales. I have therefore united the two genera."

For the separation of his *Vandellia* and *Lindernia* Haines depends only on one point, *i.e.*, the nervation of leaves as suggested by J. D. Hooker. It would have been more appropriate for him to go one step further and combine the four into a single genus, but this was to be done by Pennell, who in his Scroph. of East Temperate North America (*Monographs, Academy of Natural Sciences of Philadelphia*, No. 1, 1935) reduced *Vandellia*, *Ilysanthes* and *Bonnaya* into *Lindernia*. Pennell's remarks in this connection are worth quoting : "By the union of the four-anthered *Lindernia* All. and *Vandellia* L. with the two-anthered *Ilysanthes* Raf. and *Bonnaya* Link and Otto. is formed a large and natural genus. It is characterised by the remarkably uniform corolla (with narrow posterior lip much shorter than the widely spreading anterior lip), by similar curiously recurving anterior filaments (the proximal portion of each projecting as if it were an appendage and the filament forked although actually the process is formed by the sharp inbending of the filament) and by similar septicidal dehiscence of the capsule (that nearly always leaves the entire septum persisting as a median plate)."

The total number of species under *Lindernia* in its new and amplified form would be about 70, of which 28 are found within the boundaries of India and Burma. These are enumerated below. A modified description of the genus with a key to the species found in our area is also given.

Lindernia Allioni

Herbs, usually annual, slender, creeping or erect, branched, glabrous or pubescent, often growing in marshy places. Leaves opposite, entire or toothed, penninerved or parallel-nerved. Flowers small, sessile or pedunculate, solitary in the axils of leaves in terminal racemes, bracteolate, often deflexed in fruit. Calyx 5-toothed or 5-partite with linear segments, scarcely imbricating. Corolla-tube cylindric or somewhat enlarged above ; upper lip erect, broad, concave, emarginate or sharply 2-fid, lower lip larger, spreading, with 3 broad, subequal lobes. Stamens 4, all perfect or the posticous or the anticous pair reduced to staminodes ; filaments filiform, the posticous pair affixed to the corolla tube, the anticous pair affixed to the throat, with a tooth-like or subulate appendage at the base ; anthers subcoherent

or coherent; cells divaricate, often confluent at the apex. Style bilamellate at the apex. Ovules numerous in each cell. Capsule globose, ovoid, oblong to linear, septicidal; valves slender, entire. Seeds numerous, foveolate, rugose.

A. Capsule about equalling the calyx or shorter.

B. Leaves penninerved.
C. Flowering calyx cleft to the middle or less.
D. Pedicels about 4 times as long as the fruiting calyx .. .. .. crustacea.
D. Pedicels twice as long as the fruiting calyx or shorter.
E. Leaf blade 1·5 cm. long or less, glabrous. molluginoides.
E. Leaf blade 2–2·5 cm. long, hairy on both surfaces .. .. Hookeri.
C. Flowering calyx cleft to the base or nearly so.
D. Flowers 3–4 mm. long.
E. Non-succulent herbs, leaves petioled .. elata.
E. Succulent herbs, leaves (at least the upper) sessile.
F. Glabrous; sepals shorter than capsule. multiflora.
F. Pubescent with spreading hairs; sepals longer than capsule .. .. hirsuta.
D. Flowers 8–12 mm. long.
E. Herbs not succulent; racemes, sub-umbellate, or flowers solitary.
F. Leaves 1–1·5 cm. long, sparsely hairy.
G. Fruiting calyx equalling the capsule. hirta.
G. Fruiting calyx twice as long as the capsule .. .. .. laxa.
F. Leaves 2·5–3·5 cm. long villous on both surfaces .. .. .. mollis.
E. Herbs succulent; racemes elongate .. punctata.
B. Leaves parallel-nerved.
C. Perfect stamens 4 .. .. .. pyxidaria.
C. Perfect stamens 2.
D. Erect herb, very small and slender; corolla thrice as long as calyx .. .. minima.
D. Diffuse or creeping herb; corolla twice as long as calyx .. .. .. rotundifolia.

A. Capsule twice as long as calyx or longer.

B. Perfect stamens 4.
C. Flowering calyx cleft to the middle.
D. Flowers pedicelled .. .. .. numularifolia.
D. Flowers sessile .. .. .. sessiliflora.
C. Flowering calyx cleft almost to the base.
D. Leaves ovate, shortly petioled .. .. cordifolia.
D. Leaves linear or linear-lanceolate, sessile angustifolia.

B. Perfect stamens 2.
C. Leaves parallel-nerved.
D. Corolla 3–4 times as long as calyx .. hyssopioides.
D. Corolla twice as long as calyx or shorter. parviflora.
C. Leaves penninerved.
D. Corolla white or red.
E. Staminodes present.
F. Staminodes hairy.
G. Leaves sessile, corolla 6–7 mm. long, fruit 12–15 mm. long .. ciliata.
G. Leaves petioled, corolla 18–20 mm. long, fruit 25–30 mm. long .. ruelloides.
F. Staminodes glabrous.
G. Leaves very sharply spinous-serrate, teeth 1–1·5 mm. apart .. bractioides.
G. Leaves shallowy serrate, teeth about 3 mm. apart .. .. .. quinqueloba.
E. Staminodes absent .. .. .. estaminodiosa.
D. Corolla blue or violet.
E. Corolla 12 mm. or more long.
F. Capsule linear-subulate, leaves broadly elliptic to ovate-oblong .. .. anagallis.
F. Capsule narrowly cylindric; leaves linear or narrowly lanceolate .. verbenæfolia.
E. Corolla 6 mm. long or shorter.
F. Leaves distantly and shallowly toothed, oblong .. oppositifolia.
F. Leaves entire or nearly so, linear .. tenuifolia.

1. **Lindernia crustacea** (Linn.) F. Muell. Cens. Austral. Pl. p. 97; Pennell in Acad. Nat. Sc. Phil. Monogr. 5, (1943), p. 29.
Caparia crustacea Linn. Mant. 87.
Vandellia crustacea Bth. Scroph. Ind. 35, and in DC. Prod. X, p. 413. Hk. f. Fl. Brit. Ind. IV, 279.
Torenia varians Roxb. Fl. Ind. 111, p. 96.
Throughout India, up to 1,600 m. in the Himalayas; tropics of Old World, introduced into tropical parts of America.

2. **L. molluginoides** (Bth.) Wettst. in Nat. Pflanzen. f. iv. 3b., p. 80.
Vandellia molluginoides Bth. Scroph. Ind., p. 35 and in DC. Prod. X., p. 413. Hk. f. *loc. cit.* 279.
Burma.

3. **L. Hookeri.** (Cl.) Wettst. *loc. cit.*, p. 79; Pennell *loc. cit.* 39.
Vandellia Hookeri Cl. ex. Hk. f. *loc. cit.*, 280.
V. stemonoides Prain Bengal Pl., p. 762; Haines Bot. Bihar and Orissa, 631; non Miq.
Bihar, Chotanagpur, N. Bengal, Khasia Mts., Pegu.
L. Hookeri subsp. **kumaunensis** Pennell, *loc. cit.*, p. 30.
Kumaon.

4. **L. elata** (Bth.) Wettst. *loc. cit.*, p. 79.
Vandellia elata Bth. Scroph. Ind., p. 36 and in DC. Prod. X, p. 414 ; Hk. f., *loc. cit.*, 280.
Burma.

5. **L. multiflora** (Roxb.) Mukerjee Comb. nov.
Vandellia multiflora G. Don. Gen. Syst. IV, 549 ; Bth. in DC. Prod. X, p. 414. Hk. f. *loc. cit.*, 280.
V. erecta Bth. Scroph. Ind., p. 36, in part.
Torenia multiflora Roxb. Fl. Ind. 111, p. 96.
Bengal.

6. **L. hirsuta** (Bth.) Wettst. in Nat. Pflanzen. f. IV, 36, p. 79.
Vandellia hirsuta Bth. Scroph. Ind., p. 36, and in DC. Prod. X., p. 44. Hk. f. Fl. Brit. Ind. IV, 280.
S. India, Sikkim Terai and Bengal to Burma ; Ceylon and eastwards to Phillippine Islands.

7. **L. hirta** (Cham. and Schl.) Mukerjee comb. nov.
L. scabra (Bth.) Wettst. *loc. cit.*, 79.
Torenia hirta Cham. and Schl. in Linnæ 11, p. 571.
Vandellia scabra Bth. Scroph. Ind., p. 36, and in DC. Prod. X, p. 414, Hk, f. *loc. cit.*, p. 281.
Columnea minuta Roxb. Fl. Ind. 111, p. 98.
Southern and eastern parts of India ; S. Africa, Madagascar and tropical parts of Asia.

8. **L. laxa** (Bth.) Mukerjee comb. nov.
Vandellia laxa Bth. Scroph. Ind., p. 36 and in DC. Prod. X., 414, Blatter and Hallb. in Bomb. Nat. Hist. Soc. XXV (1918), p. 416.
V. scabra var laxa Hk. f. *loc. cit.*, p. 281.
Vingrola-Konkan ; High wavy mts.-Madura Dist., Guindy Madras (Prov.).

9. **L. mollis** (Bth.) Wettst., *loc. cit.*, p. 79.
Vandellia mollis Bth. Scroph. Ind., p. 37 and in DC. Prod. X., p. 414, Hk. f., *loc. cit.*, p. 281.
Sikkim, Assam, Burma, S. China.

10. **L. punctata** (Prain) Mukerjee comb. nov.
Vandellia punctata Prain in Journ. Roy. As. Soc. Bengal LXXII (1903), p. 19.
Shan Hills, Port, Stedman, Taungyi.

11. **L. pyxidaria** All. in Misc. Taurin 3 (1766) 178, tab. 5 ; Linn. Mant. Pl. 2 ; (1771) 252 ; Pennell in Acad. Nat. Sc. Phil. Monograph, 5 (1943), p. 25.
L. erecta Botanii ?
Vandellia erecta Bth. Scroph. Ind. 36, and in DC. Prod. X., p. 415, Hk. f. *loc. cit.*, 281.
Gratiola integrifolia Roxb. Fl. Ind. i, 137.
Throughout India upto 1,700 m. in the Himalayas ; eastwards to Polynesia, and westwards to Europe.

12. **L. minima** (Bth.) Mukerjee comb. nov.
Ilysanthes minima Bth. in DC. Prod. X, p. 420, Hk. f. Fl. Brit. Ind. IV, p. 284.
S. India.

13. **L. rotundifolia** (Linn.) Mukerjee comb. nov.
Gratiola rotundifolia Linn. Mant., p. 274 ; Roxb. Cor. Pl. iii, 3, t. 204, and Fl. Ind., p. 137.
Ilysanthes rotundifolia Bth. in DC. Prod. X, p. 420 ; Hk. f. *loc. cit.*, 254.
S. India, Ceylon, Mauritius and Madagascar.

14. **L. numularifolia** (Don) Wettst. in Nat. Pflanzenf. IV, 3b, p. 97, Pennell in Acad. Nat. Soc. Phil. Monogr. 5, (1943), p. 29.
Vandellia numularifolia Don. Prod. Fl. Nep., p. 86 ; Bth. in DC. Prod. X, p. 416 ; Hk. f. *loc. cit.*, p. 282.
Subtropical Himalayas from Kashmir to the Mishmi Hills, S. India, Chotanagpur, Assam and hills of Burma.

15. **L. sessliflora** (Bth.) Wettst. *loc. cit.*, p. 79.
Bonnaya micrantha Blat. and Hallb. in Journ. Bomb. Nat. Hist. Soc., XXV (1918), p. 417.
Vandellia sessiliflora Bth. Scroph. Ind., p. 37 and in DC. Prod. X, p. 416, Hk. f. *loc. cit.*, 282.
Subtropical Himalayas, Assam, Burma, S. Ind ia.

16. **L. cordifolia** (Colsm.) Merrill Enum, Phillp. Pl. III, p. 437 ; Pennell, *loc. cit.*, p. 30.
Gratiola cordifolia Colsm. Prod. Desc. Grat., p. 15.
Vandellia pedunculata Benth Scroph. Ind. 37 and in DC. Prod X, 416, Hk f., *loc cit.* 282.
Vandellia cordifolia G. Don, Gen. Syst. IV, p. 549 ; Haines Bot. Bihar and Orissa, p. 633.
V. cerastoides Collet and Hemsl. in Journ. Linn. Soc., Vol. XXVIII (1890), p. 100.
Throughout India, Ceylon, Malaya and eastwards to Australia.

17. **L. angustifolia** (Bth.) Wettst., *loc cit.*, 79, Pennell, *loc. cit.*, p. 31.
Vandellia angustifolia Bth. Scroph. Ind., p. 37, and in DC. Prod. X, p. 417, Hk, f., *loc. cit.*, p. 282.
V. verbenaefolia Haines Bot. Bih. and Orissa, p. 634, in part.
Subtropical Himalayas, Kumaon, Nepal, Sikkim, Chotanagpur, Assam, Burma.

18. **L. hyssopioides** (Bth.) Haines, *loc. cit.*, p. 635.
Ilysanthes hyssopioides Bth. in DC. Prod. X, p. 419 ; Hk. f., *loc. cit.*, p. 283.
S. India, Chotanagpur (Sarguja), Assam, Burma (?) ; Ceylon, Malaya, China.

19. **L. parviflora** (Roxb.) Haines., *loc. cit.*, p. 635, Pennell, *loc. cit.*, p. 29.
Gratiola parviflora, Roxb. Cor. Pl. III, p. 3, t. 204 and in Fl. Ind., I, p. 140.

Ilysanthes parviflora Bth. in DC. Prod. X, p. 419, and Scroph. Ind. 34 ; Hk. f., *loc. cit.*, 283.

Throughout India upto 1800 m. in the Himalayas ; Siam, Malaya peninsula ; Trop. Africa.

20. **L. ciliata** (Colsm.) Pennell in Journ. Arn. Arb. Vol. 24 (1943), p. 253 ; et. in Monog. Acad. Nat. Soc. Phil. No. 5, p. 32.
Gratiola ciliata Colsm. Prod. Desc. Grat., p. 14.
Bonnaya brachiata Link and Otto, Icon. Pl. Select. 25, t. 11 ; Hk. f., *loc. cit.*, p. 284.
Vandellia brachiata Haines, *loc. cit.*, p. 632.

Throughout India, up to 1,600 m., in the Himalayas ; Ceylon, Malaya and eastwards to the Philippine Islands.

21. **L. ruelloides** (Colsm.) Mukerjee comb. nov.
Gratiola ruelloides Colsm. Prod. Desc. Gratiola 12, Roxb. Fl. Ind., p. 140.
Bonnaya reptans Spr. Syst. 1, p. 410 ; Hk. f., *loc cit.*, p. 284.
Ilysanthes reptans Urban Berl. Deutsch. Bot. Ges. 11, p. 436.
L. ruelloides O. Ktze Gen. Pl., p. 462.

Nepal, Sikkim, Assam and Burma ; South India, Java, Phillipine Islands.

22. **L. bracteoides** (Blat. and Hallb.) Mukerjee Comb. nov.
Bonnaya bractoides Blat. and Hallb. in Journ. Bomb. Nat. Hist. Soc. XXV (1916), p. 416.

Common in Abu Mts.

23. **L. quinqueloba** (Blat. and Hallb.) Mukerjee comb. nov.
Bonnaya quinqueloba Blatt. and Hallb. in Journ. Bomb. Nat. Hist. Soc. XXV (1918), p. 417.

24. **L. estaminodiosa** (Blat. and Hallb.) Mukerjee comb. nov.
Bonnaya estaminodiosa Blat. and Hallb. in Journ. Bomb. Nat. Hist. Soc. XXV (1918), p. 416.

Mahim, Bombay Island.

25. **L. anagallis** (Burm.) Pennell in Journ. Arn. Arb. Vol. 24 (1943), p. 252.
Ruellia anagallis Burm. Fl. Ind., p. 135.
Bonnaya veronicæfolia Spr. Syst. I. 14 ; Hk. f. *loc. cit.* 285.
Vandellia veronicæfolia Haines, *loc. cit.*, 633.
L. antipoda Alston in Trim. Handb. Fl. Ceylon VI ; suppl., p. 214.

Throughout India ; eastwards to the Philippine Islands.

Var grandiflora (Spr.) Mukerjee comb. nov.
B. grandiflora Spr., *loc. cit.*, p. 41.
B. veronicæfolia var *grandiflora* Hk. f., *loc. cit.*, 285.

Throughout India.

26. **L. Verbenæfolia** (Colsm.) Pennell, *loc. cit.*, p. 131.
Gratiola verbenæfolia Colsm. Prod. Desc. Grat., p. 8.
Bonnaya verbenæfolia Bth. in DC. Prod. X, p. 421.
B. veronicæfolia var. verbenaefolia Hk. f., *loc. cit.*, p. 295.
Vandellia verbenæfolia Haines, *loc. cit.*, p. 634.

Upper Gangetic Plain, Bengal, Burma, S. E. Asia.

27. **L. oppositifolia** (Linn.) Mukerjee comb. nov.
Gratiola oppositifolia Linn. Sp. Pl. ed. Willd., Vol. I, p. 105; Roxb. Cor. Pl. II, p. 30, t. 155.
Bonnaya oppositifolia Spr. Syst. 1, p. 41, Benth. in DC. Prod. X, p. 421 ; Hk. f., *loc. cit.*, 286.
Vandellia oppositifolia Haines Bot. Bihar and Orissa, p. 634.
Ilysanthes oppositifolia Urban in Berl. Deutsch. Bot. Ges. II (1884), p. 435.
S. India, Manbhum.

28. **L. tenuifolia** (Vahl.) Alston in Trim. Fl. Ceylon, VI, Suppl., p. 214.
Gratiola tenuifolia Vahl. Enum. 1, p. 95.
Bonnaya tenuifolia Spr. Syst. 1, p. 42 ; Bth. in DC. Prod. X, p. 422 ; Hk. f., *loc. cit.* 286.
Ilysanthes tenuifolia Haines Bot. Bihar and Orissa, p. 634.
Bengal to Burma, S. India ; Ceylon and China.

AN ANATOMICAL STUDY OF *TILIACORA ACUMINATA* MIERS.

BY BALWANT SINGH

Dacca University

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1. INTRODUCTION

Tiliacora acuminata (Lam.) Miers. (= *T. racemosa* Colebr.) is a dicœcious evergreen climber of the Menispermaceæ, which is known to occur in various parts of India, Burma, Ceylon and Malaya (Brandis, 1874).

The plant is usually seen near hedges and bushy clumps and it frequently climbs over forest trees. The young shoots are green but the older ones are covered with a thin brownish layer of cork. The lenticels are few in number and elongated along the axis of the stem with their margins slightly raised above the general surface. A cross-section of an old stem presents an extremely abnormal appearance, for, instead of a single ring of vascular tissue as is met with in most dicotyledons, we find here a series of concentric rings or large arcs of bundles separated by tangential bands of parenchyma, while in between the bundles of each ring lie the wide interfascicular rays (Figs. 1, 2*a*). Eighteen such rings were counted at the base of a stem, about 8·8 cm. in diameter. Owing to the abundance of fibrous cells in the wood, the stems show a high degree of flexibility and are consequently used in many places for thatching and basket work.

The full grown leaves are 16·5 to 19·0 cm. by 9 to 11 cm. in size, 3- to 5-nerved at the base, ovate, acuminate and cordate, truncate or rounded at the base with undulate margins, and glabrous except on the lower side of the midrib. The petiole is 2·5 to 3·5 cm. in length with its base somewhat flattened and twisted so as to serve as a hook for helping the plant in attaching itself to its support.

The root system consists of a woody tap-root with many branches. The anomaly seen in the stem is also present here (Figs. 2*b* and *c*) and 7 rings of vascular tissue were seen in a root about 4·2 cm. thick. According to Roxburgh (1832, Vol. 3, p. 816) the root is used as a cure for snake-bite.

2. PREVIOUS WORK

A reference to the works of Solereder (1908) and Pfeiffer (1926) shows that anomalous secondary growth has so far been noted in the family Menispermaceæ in the stems and roots of *Abuta*, *Chondrodendron* *Cissampelos*, *Clypea* and *Cocculus* and in the stems only of *Anamirta*, *Anomospermum*, *Chasmanthera*, *Hyperbæna*, *Jateorhiza*, *Menispermum*,

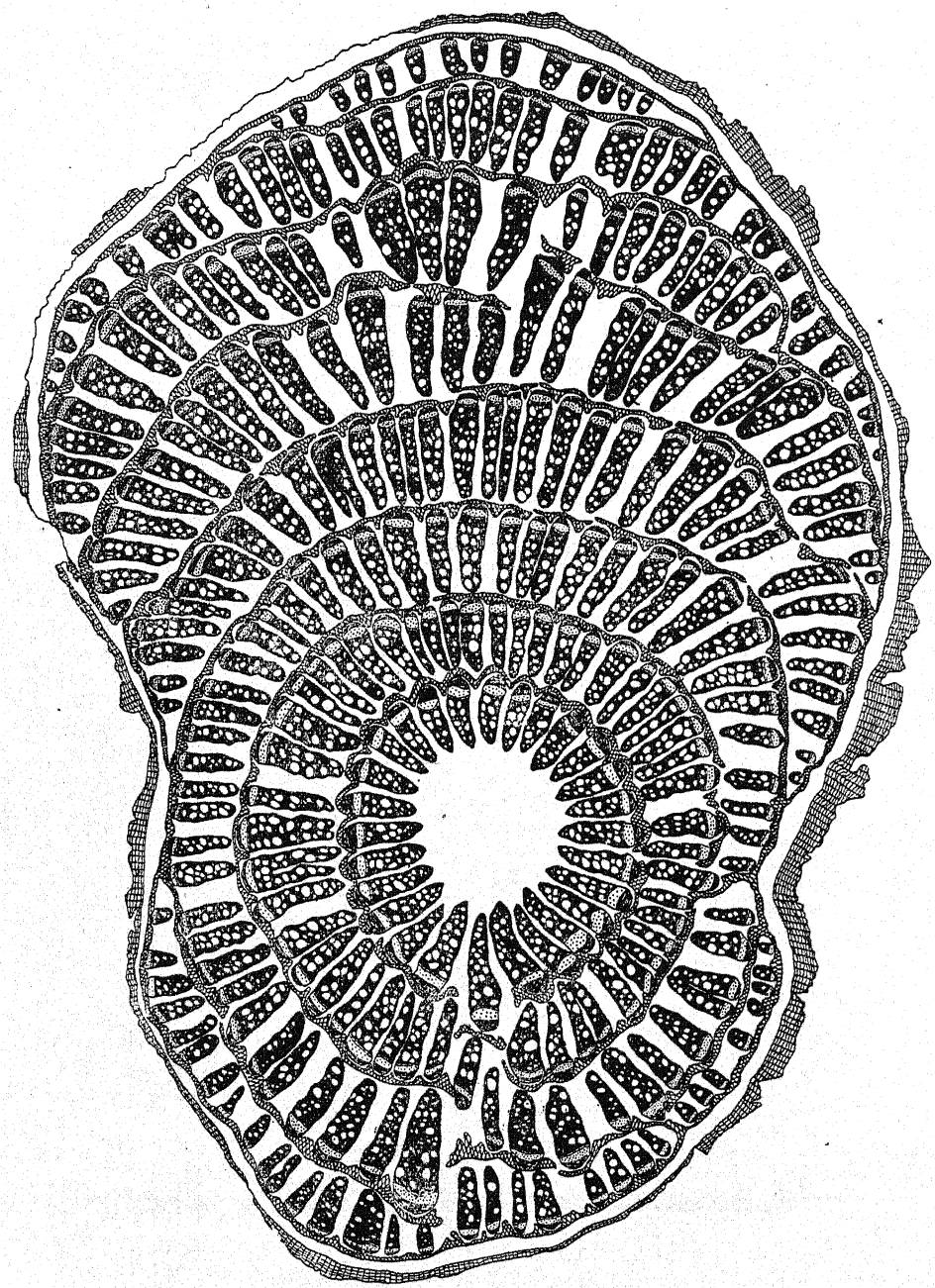

Fig. 1. An outline diagram of t.s. of stem showing anomalous secondary growth. × 9.

Pachygone, *Pericampylus* and *Stephania*. Recently Santos (1931) described it in the stems of *Anamirta cocculus* and *Archangelisia flava* and Maheshwari (1935) has reported it in the stem of *Tiliacora*. No detailed work on the anatomy of the last named plant has, however, yet appeared and this study was undertaken in the hope that it might lead to a clearer idea of the origin of the supernumerary cambial rings which form such a characteristic feature of the stem and root in several plants of the family.

3. Material and Methods

The greater part of the material used in this study was collected locally and fixed in formalin-acetic-alcohol. This was dehydrated, infiltrated and imbedded in paraffin according to the usual methods. Older pieces of the stem and root were first treated with dilute HF. Section were cut between 12 and 25 μ and stained with Safranin and Fast Green. In a few cases Bismarck Brown and Iron-hæmatoxylin were also used for comparison. The oldest stems and roots were cut fresh on a sliding microtome. Mounts of macerated material were also prepared for a study of the shapes and characteristics of the individual cells and the strip method of Priestley, Scott and Malins (1933) was used to make some preparations of the tissues adjacent to the primary cambium[1].

4. The Stem

Primary Structure.—A cross-section of the stem is roughly circular with some undulations in the younger stages which smoothen out afterwards. The *epidermis* consists of a layer of moderately cutinised cells with occasional stomata. Epidermal hairs are few, occurring only in the youngest regions. They are invariably 2-celled with a short stalk cell which is thick-walled and urn-shaped and a terminal cell which is long, slender and thin-walled. The deposition of cutin is usually seen only on the outer walls but occasionally it extends even to the radial and inner walls of some epidermal cells.

The *cortex* consists of 3–4 layers of collenchymatous cells followed by 4–10 layers of more or less tangentially elongated parenchyma. Chloroplasts are present in both but are more numerous in the outer layers, particularly in the substomatal portions. Solitary thick-walled cells of a fibrous nature are also seen in the cortex but only occasionally and without any definite plan in their arrangement or distribution. "Bitter principle sacs" such as have been noted in *Tinospora* by Santos (1928) were seen in only a few sections.

An *endodermis* is not clearly distinguishable. Many cells of the innermost layer of the cortex which are in contact with the band of sclerenchyma contain rectangular or rhomboidal crystals of calcium oxalate. Due to the lack of a distinct endodermal layer it is not possible to set off the tissues of the stele from the cortex with any

[1] The designation "primary cambium" is used here to distinguish the cambium of the normal ring of vascular bundles from the subsequently formed "secondary" or "extrafascicular" meristems.

certainty, but by analogy with other menispermaceous stems (see Santos, 1928, 1931), the crescent-shaped arches of sclerenchymatous cells (Fig. 3) which lie outside the vascular ring may be said to belong to the pericycle. The outer of these cells are small and thick-walled and have a narrow lumen while the inner are larger and do not show such a pronounced thickening of their walls. In macerated material the fibres are seen as long and tapering cells with simple slit-like pits on their walls. Next to the sclerenchyma comes a thin-walled parenchymatous tissue, the inner pericycle, composed of 3–5 layers of closely packed polygonal cells which become greatly compressed and flattened in older stages. It is of interest to note that although originally there is a continuous cyclinder of pericyclic fibres, this later becomes ruptured as a result of the increase in girth of the stem; the interfascicular parenchyma cells intrude however into the gaps and become converted into stone cells thus repairing the broken cylinder.

The *vascular bundles* vary in number from about 20 to 40 and are separated by the medullary rays which are usually 4 to 8 cells wide and consist of radially elongated cells with small intercellular spaces. An increase in the number of bundles may be brought about either by the splitting of the original bundles owing to the formation of secondary vascular rays, or by the production of secondary strands in the interfascicular region. It is frequently seen that the bundles on one side of the stem are larger than those on the opposite side, thus giving it an eccentric appearance which becomes still more pronounced in subsequent stages.

The *pith* occupies a large proportion of the space in a young stem. The cells are large and spherical with prominent inter-cellular spaces at the angles. The peripheral cells are more thick-walled than those in the centre and form a sort of perimedullary zone as is seen in many other dicotyledons (Eames and Macdaniels, 1925, p. 99). Starch grains and calcium oxalate crystals are common.

Secondary Growth.—At a very early stage a fascicular cambium differentiates in each bundle and gives rise to the secondary tissues in the normal manner. There is however no definite inter-fascicular cambium, although some stray tangential divisions are occasionally seen in the cells of the medullary rays. A glance at Figs. 3 and 4 will show that the sclerenchymatous cells of the pericycle invaginate so deeply into the rays that the formation of a continuous cambial ring is hardly possible.

As a result of the activity of the fascicular cambium the primary phloem and afterwards even the older secondary phloem cells become crushed and obliterated to form a densely staining cap over the vascular bundles (Fig. 4). It is worthy of note that the sieve plates are not transverse to the longitudinal axis of the sieve tubes but lie at angle of about 45°.

The secondary xylem consists of vessels, fibre-tracheids and wood parenchyma. The vessels are often large enough to be made out with the naked eye. They occur either singly or in groups of two to four. The vessel elements have simple rounded perforations and

some of them are provided with tails at one or both ends. Their walls show alternately arranged bordered pits with slit-like apertures which are just included in the border. Fibre-tracheids are abundant and have thick walls with numerous bordered pits having slit-like apertures. The xylem parenchyma cells may be short or elongated and have simple pits with rounded, oval or slit-like openings. The ray cells have simple pits except when they are in contact with vessels or fibre-tracheids in which case the pits are bordered. In tangential sections and strip preparations the rays appear as large more or less homogeneous boat-shaped areas whose lateral cells are particularly rich in calcium oxalate crystals. Short thin-walled "disjunctive parenchyma"[2] cells are also seen although only occasionally. These are wider, having simple rounded or oval pits and short tubular processes.

The older stems usually become eccentric owing to more active growth on one side (Fig. 1). According to Priestley and Tong (1927), Haberlandt (1914, p. 676) and others, such eccentricity in growth may be caused by gravity, mechanical strain, light, moisture, nutrition, temperature, wind, etc., having an unequal influence on the two opposite sides of the stem or root of plants. I have not been able to determine the cause of the eccentricity but the lower side is usually the one which shows more active growth in *Tiliacora* (*cf.* Maheshwari, 1930, on *Boerhaavia*).

Cork formation begins only after a fair amount of secondary growth has taken place. The subepidermal cells undergo some radial elongation followed by tangential divisions which result in the demarcation of the phellogen. The cork cells produced on the outside are more or less rectangular in cross-section and lie in distinct radial rows. The phelloderm, which is produced on the inside, is very narrow and with increase of secondary growth the shape and arrangement of its cells become distorted. Crystals of calcium oxalate are of frequent occurrence and some of the cells become converted into stone cells.

Anomalous Secondary Growth.—The primary cambium becomes inactive after some time (Fig. 4). When this happens, the parenchymatous cells (Fig. 5) lying just outside the sclerenchymatous bands show a radial elongation (Fig. 6) and soon begin to undergo some periclinal divisions, as a result of which the inner cortex becomes converted into a meristematic zone composed of about 4 to 10 layers of cells. A few of the outermost layers of this tissue usually remain undifferentiated and afterwards give rise to another ring of vascular tissue as we shall see later. The next 1 to 3 layers give rise to a ring of stone cells[3] (Fig. 7) and in the remaining portion of the meristem further periclinal and anticlinal divisions now take place at a number of points resulting in the differentiation of groups of phloem cells (Fig. 8) followed

[2] This name has now replaced the "conjugate parenchyma" of older authors (see Record, 1933, p. 9).

[3] The radial series cannot always be traced outside the stone cells due to the distortion brought about by the inward pressure of the periderm and the outward pressure of the secondary vascular tissue.

internally by some xylem elements (Fig. 9). Some intervening cells between the xylem and phloem continue to retain their meristematic character and form the intrafascicular cambium. As mentioned before, the anomalous growth at first starts in short segments, but it extends laterally to form fairly large arcs of secondary vascular tissue which abut upon the inner ring of bundles (Fig. 10).

Secondary activity in the newly formed ring of bundles is short-lived but another meristem soon arises from the residual cells of the previous one which had been left undifferentiated at its outer edge. The differentiation of stone cells and vascular tissues in this tertiary ring takes place in a manner similar to that already described for the second ring. The subsequent rings originate from the outer cells of their predecessor as in the case of the second and third rings. The production of these supernumerary rings continues for a long time, resulting in the marked polycyclic condition already noted in the beginning of this paper.

The vascular bundles of the anomalous rings are very similar to those of the primary ring except that the former lack all primary xylem and phloem and that the primary ring has on its outside an undulate band composed mainly of fibrous cells while those formed later are overarched by one to three layers of stone cells. Owing to the presence of these sclerenchymatous elements outside every ring of vascular tissue, the pluriseriate nature of the stem is very clear even to the naked eye. The xylem vessels are often found to contain tyloses which are at first thin and bladder-like but later become pitted.

5. The Leaf

Petiole.—A cross-section of the petiole presents a more or less broad crescent-shaped outline. The cuticular deposit on the epidermis extends even to the radial walls of its cells as in the case of the stem. Epidermal hairs are of the same kind as seen in the stem and are continued on the lower side of the lamina. Next to the epidermis come 3 to 6 layers of compactly arranged collenchymatous cells with chloroplasts. In the inner cortex, which consists mainly of parenchymatous cells, there are also some isolated sclerenchymatous elements with thick walls and a broad lumen. Three leaf traces enter the petiole, all more or less completely surrounded by fibrous sheaths. The median trace divides immediately into three bundles and the other two also branch and anastomose, thus resulting in a total of about 8 to 12 strands arranged in a semicircle. This differs from the condition in *Anamirta*, *Menispermum*, *Cocculus* (Solereder, 1908, p. 41) and *Tinospora* (Santos, 1928) where the bundles are arranged in a circle and the petiole has a more or less rounded outline in a cross-section.

The pith consists of fairly large spherical cells with small intercellular spaces. Many of the cells contain calcium oxalate dust.

Lamina.—The leaf is bifacial. In a cross-section of the lamina the upper epidermis is seen to consist of tabular or squarish cells whose outer walls are slightly convex but not so heavily cutinised as those of the stem or petiole. The mesophyll consists of a single layer of palisade

tissue followed by the irregularly shaped cells of the spongy parenchyma which are interspersed with large air spaces. Each vein consists of a small vascular bundle surrounded by a fibrous sheath which extends both inward and downward as to some close to the epidermal layers. The cells of the sheath occasionally show small rhomboidal crystals of calcium oxalate. In some plants of the family, Solereder (1908, p. 815) and Santos (1928) record the presence of large rhomboidal crystals in the epidermal layers, which serve as a 'regular armour' : but I was unable to find them in either the lower or the upper epidermis of *Tiliacora acuminata*. Stomata were seen only on the lower surface.

The midrib is conspicuous on account of the local convexity of the leaf tissues in this region. As seen in a transverse section the epidermal cells on both sides of the midrib are usually polygonal and more thick-walled than those on the sides. Just below the epidermis, on either side of the lamina, there are 1 to 3 layers of collenchymatous cells those on the lower side being somewhat larger. They frequently contain crystals of calcium oxalate. Next to the collenchymatous tissue is the parenchyma with occasional stone cells which are more frequent in the basal part of the leaf.

The number of vascular bundles composing the midrib depends on the size of the leaf and the distance from the base at which the section is taken. The largest number (about 13) is seen at the base of the lamina, from where they pass off towards the right and left until there is only one bundle left in the distal portion of the midrib.

6. THE ROOT

Primary Structure.—A cross-section of the young root is more or less circular in outline and shows the usual piliferous layer, cortex and stele. Suberisation of the outer walls of the epidermis begins soon after the root hairs have ceased to function and extends even to layer below it. The cortex consists of about 3 to 6 layers of large polygonal cells which are often full of fungal hyphæ[4] (Fig. 11). The innermost layer forms an indistinct endodermis, sometimes easily recognisable by the Casparian strips on its radial walls which are more easily seen opposite to the primary phloem strands. Inside the endodermis there is a single layer of pericycle. The stele is usually diarch but sometimes triarch, and the primary xylem elements meet in the centre to form a "xylem plate" (Figs. 11, 13). It is only in a few roots that some small parenchymatous cells resembling those of a pith were seen in the centre but even these become lignified in later stages. In some cases they were found to contain starch grains. Some of the cells immediately outside the primary phloem and perhaps belonging to it become lignified and form fibres.

Secondary Structure.—Secondary growth begins early by the usual differentiation of cambial segments below the primary phloem bundles

[4] I am indebted to Dr. B. B. Mundkur (New Delhi) and Dr. K. Bagchee (Dehradun) for the information that a mycorrhiza is so far unknown in the Menispermaceæ.

and their subsequent extension resulting in a complete cambial cylinder. The secondary xylem elements extend so close to the primary xylem that in later stages it is difficult to demarcate the latter. Opposite to the primary xylem groups the cambium cuts off only parenchymatous tissue resulting in two (three, when the root is triarch) broad medullary rays, which divide the secondary vascular tissues into large semicircular segments. Very soon additional vascular rays originate inside each segment which is consequently split up into separate bundles of which as many as 15 may be seen in later stages.

The structure of the secondary vascular bundles is very similar to those of the stem. Tyloses are frequently present in the vessels. As secondary growth proceeds, the primary phloem cells become flattened and crushed, and a one to two-layered ring of sclerenchymatous elements appears at the periphery of the secondary vascular bundles.

Very soon after the vascular cambium has begun its activity a phellogen differentiates in the pericycle. It cuts off 7–10 layers of phellem towards the outside but much less of phelloderm. Here and there inside the former are seen some thick-walled elements which have a mechanical function. With the development of the cork, all the tissues outside it (*i.e.*, the cortex and the epidermis) die and are sloughed off.

Anomalous Secondary Growth.—The abnormal growth starts in the roots from the pericyclic cells internal to the phellogen, which first elongate radially and then divide periclinally to form a zone of meristematic cells, 8 to 10 cells wide. As in the stem, isolated patches of phloem and xylem separated by a cambium are differentiated inside it. On the outside of the meristematic zone there differentiates a layer or two of sclerenchymatous cells which often contain calcium oxalate crystals and further outward (as in the stem) a few layers of the meristem are left over to form the precursors of the next anomalous ring. Other rings (Fig. 12) are produced similarly in centrifugal succession from the residual cells of the previous meristem left over immediately outside the layer of stone cells.

7. Discussion

Anomalous thickening in plants, characterised by the production of additional complete or partial extrafascicular cambial cylinders, is observed in lianes as well as non-lianes. As regards the modes of origin of the anomalous rings, Solereder (1908, p. 1164–65) divides the dicotyledonous families into two groups :—

(i) Those in which the secondary meristem arises either in the inner cortex or occasionally even in the endodermis : Capparideæ, Caryophylleæ, Compositæ, Cucurbitaceæ, Menispermaceæ, Plumbagineæ Umbelliferæ and Verbenaceæ.

(ii) Those in which the place of origin lies deeper, either in the pericycle or even in the phloem of the original vascular ring : Amaranthaceæ, Ampelidaceæ, Bignoniaceæ, Buxaceæ, Cæsalpinieæ,

Candolleaceæ (?), Capparideæ, Chenopodiaceæ, Compositæ, Convolvulaceæ, Cucurbitaceæ, Dilleniaceæ, Euphorbiaceæ, Ficoideæ, Hippocrateaceæ, Icacineæ, Illecebraceæ, Labiatæ, Loranthaceæ, Nyctaginaceæ, Olacineæ, Phytolaccaceæ, Plumbagineæ, Polygaleæ, Rhamneæ (?), Rubiaceæ and Sapindaceæ.

In addition to the above families Pfeiffer (1926) mentions the same abnormality in the Acanthaceæ, Vochysiaceæ, Cruciferæ, and with some doubt in the Loganiaceæ, and Stylidiaceæ. A similar anomaly is met with among the Gymnosperms in the Cycadaceæ and Gnetaceæ.

As regards the Menispermaceæ, Maheu (quoted in Solereder, 1908, p. 818) reported that the anomalous rings may originate in any of the following tissues :—

(*a*) Cortex (*Menispermum*).

(*b*) Endodermis (*Abuta rufescens*, *Chondrodendron tomentosum*, *C. platyphyllum* and *Cocculus laurifolius*).

(*c*) Pericycle (*Cocculus læba*, *Cissampelos pareira*).

(*d*) The region immediately external to the phloem of the normal ring of bundles (*Abuta selloana*, *Anomospermum grandifolium*, *Cocculus platyphyllus* and *Cissampelos mauritiana*).

Pfeiffer (1926, p. 164) remarks however that Maheu's observations, which were made almost entirely on herbarium material, are not quite reliable, and suggests further work on fresh material. The only recent paper on the subject is by Santos (1931) who found that in the stems of *Archangelisia flava* and *Anamirta cocculus* the abnormal rings are formed by successively produced cambial layers differentiating in the primary cortex. Should this be so and the cambia continue to arise from the cortical cells in centrifugal succession, a time will eventually arrive when the cortex would be used up altogether. In *Tiliacora acuminata* only about 12–14 layers of cells are found in the primary cortex, but a cross-section of a thick stem already showed as many as 17 anomalous rings and there was no indication that the abnormal growth had come to an end. It was this that prompted me to make a closer study of the origin of the anomaly and compare my observations with those of other recent authors. In the stem of *Boerhaavia diffusa* (Maheshwari, 1930) and the root of *Beta vulgaris* (Artschwager, 1926), the supplementary rings of vascular tissue do not arise *de novo*, but their origin has been traced back to the normal cambial ring. Joshi (1931, 1937) supported this in the case of *Alternanthera sessilis* and other Amarantaceæ and Chenopodiaceæ and there seems to be no doubt that the supernumerary vascular rings found in the Centrospermales are in direct lineage with the first extrafascicular cambium.

My observations on *Tiliacora acuminata* correspond with those of the above authors and are at variance with the results obtained by Santos (1931). Briefly, there is at first a single ring of vascular bundles in the stem, separated from each other by broad medullary rays. The cambium in these bundles becomes inactive after a time and a new extrafascicular cambium arises in the cells lying immediately outside the pericycle. This gives rise to a broad zone of meristematic cells

a few layers of which lying at the periphery remain undifferentiated for the present ; the next 1–3 layers give rise to stone cells and the rest undergo further divisions to produce a ring of secondary vascular bundles. Now, after a brief period of secondary growth in these bundles, the two or three layers of undifferentiated cells left over outside the ring of stone cells begin active divisions and behave in the same way as the first meristem. This process repeats itself several times, giving a pluriseriate character to the stem. The same anomaly occurs in the root with this difference that here the secondary cambium and the phellogen both originate in the pericycle.

It is worthy of note that two different types of secondary growth occur in the family Menispermaceæ. A few genera like *Tinospora* (Santos, 1928) show only the normal type, while others (see list on p. 2) are characterised by the formation of successive rings of centrifugally differentiated vascular bundles. A study of the published data on the habit of the plants seems to indicate that the difference is not related to the environment.

Whether this condition is the result of parallel development or whether one of the two conditions is derived from the other is therefore difficult to say in the present state of our knowledge of the morphology and cytology of the plants belonging to this family.

8. Summary

1. A cross-section of the young stem of *Tiliacora acuminata* shows a normal ring of vascular bundles separated by broad medullary rays. An interfascicular cambium is inactive or absent. The pericycle consists of a ring of sclerenchymatous elements made up of arcs of fibres capping each vascular bundle. A well-differentiated endodermis is absent.

2. Older stems show a series of concentric rings of vascular bundles (18 rings were counted in a stem 8·8 cm. thick), separated by wide rays and tangential bands of stone cells and thin-walled parenchyma.

3. Sections cut at different levels of the stem show that after a while the normal fascicular cambia of the primary ring of bundles become inactive and an extrafascicular meristem originates from the cortical cells lying immediately outside the pericycle. A few of the outermost layers of this meristem remain undifferentiated, the next 1–3 layers give rise to stone cells, and the rest differentiate into a secondary ring of vascular bundles separated by broad rays of parenchymatous cells. After a time the activity of the cambia in these bundles also ceases and a second meristem arises from the undifferentiated cells of the first one left over outside the stone cells. This process is repeated so as to give rise to a large number of anomalous rings of vascular bundles arising in centrifugal succession.

4. There is a well-marked eccentricity in the stems, the lower side showing greater growth and a larger number of anomalous rings (or their segments) than the upper.

5. Three leaf-traces enter the petiole, but they further split and anastomose in their course so that there is no constancy in their number and 8 to 12 bundles may be seen in a cross-section of the petiole.

6. The lamina shows the usual structure of a mesophytic dicotyledonous leaf with the stomata confined to the lower surface. The midrib has about a dozen bundles at the base but these pass out right and left and ultimately only a single bundle is seen at its distal end.

7. The root is usually diarch but sometimes triarch. The cortical cells are often full of fungal hyphæ. Cork formation takes place in the pericycle, and after the production of the first and normal ring of secondary vascular tissues a new cambium arises from the pericyclic cells internal to the periderm and gives rise to an additional ring of vascular bundles outside the first one. As in the stem, there is a centrifugal succession of such rings of vascular tissue which gives a pluriseriate appearance to the mature roots.

8. Crystals of calcium oxalate are found in all parts of the plant.

9. ACKNOWLEDGMENTS

My grateful thanks are due to my teacher Dr. P. Maheshwari for his valuable guidance and keen interest in my work. In the collection of the material and in many other ways I received the ungrudging help and co-operation of Mr. S. K. Sen, Curator of the University Herbarium and Garden. To Prof. B. Sanhi (Lucknow), Dr. A. C. Joshi (New Delhi) and Mr. K. R. Rao (Dacca), I am indebted for reading the manuscript and examining some of my preparations.

LITERATURE CITED

Artschwager, E. F. (1926) .. "Anatomy of vegetative organs of Sugar Beet," *Jour. Agric. Res.*, **33**, 143–76.

Brandis, D. (1874) .. *The Forest Flora of North-West and Central India*, London.

de Bary, A. (1884) .. *Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns*, Eng. Trans., Oxford.

Eames, A. J. and MacDaniels, L. H. (1925) *An Introduction to Plant Anatomy*, McGraw Hill, New York.

Haberlandt, G. (1914) .. *Physiological Plant Anatomy*, Eng. Trans., London.

Joshi, A. C. (1931) .. "Contribution to the anatomy of the Chenopodiaceæ and Amarantaceæ. Anatomy of *Alternanthera sessilis* R. Br.," *Jour. Ind. Bot. Soc.*, **11**, 213–31.

———— (1937) .. "Some salient points in the evolution of the secondary vascular cylinder of Amarantaceæ and Chenopodiaceæ," *Amer. Jour. Bot.*, **24**, 3–9.

Maheshwari, P. (1935) .. "Anomalous secondary growth in the stem of *Tiliacora racemosa*, Colebr.," *Proc. Ind. Sci. Cong. 22nd Session. Calcutta.*

———— (1930) .. "Contributions to the morpho'ogy of *Boerhaavia diffusa* (II)", *Jour. Ind. Bot. Soc.*, **9**, 42–61.

Pfeiffer, H. (1926) .. "Das abnorme Dickenwachstum," In Linsbauer's *Handbuch der Pflanzenanatomie*, Borntträger, Berlin.

Priestley, J. H. and Tong, D. (1927) "The effect of gravity on cambial activity in trees," *Proc. Leeds Phil. Soc.*, **1**, 199-208.

———, Scott, L. I. and Malins, M. E. (1933) "A new method of studying cambial activities," *ibid.*, **2**, 365–74.

Record, S. J. (1933) .. "Glossary of terms used in describing woods," *Tropical Woods*, No. 36, 1–12.

Roxburgh, W. (1832) .. *Flora Indica.*

Santos, J. K. (1928) .. "Stem and leaf structure of *Tinospora rumphii* Boerlage, and *Tinospora reticulata* Miers," *Philippine Jour. Sci.*, **35**, 187–208.

———— (1931) .. "Anomalous stem structure in *Archangelisia flava* and *Anamirta cocculus* from Philippines," *ibid.*, **44**, 385–407.

Solereder, H. (1908) .. *Systematic Anatomy of the Dicotyledons*, Eng. Trans., Oxford.

EXPLANATION OF PLATES

PLATE VII

Fig. 2*a*. Transversely cut surface of a thick stem showing the supernumerary rings of vascular tissues; *b* & *c*. Cross and longitudinal sections of the root to show the same anomaly. ×½.

Fig. 3. T.s. of portion of a young stem. ×70.

Fig. 4. T.s. of a part of stem showing the approximate stage after which anomalous growth starts. ×70.

PLATE VIII

Fig. 5. T.s. of a portion of young stem showing the epidermis, cortex, and the sclerenchymatous pericycle. ×290.

Fig. 6. Part of an older stem showing the origin of extra-fascicular cambium. Note the radial elongation and periclinal divisions in the inner cortical cells. ×290.

Fig. 7. The meristematic zone is well established and a band of stone cells is being differentiated at its periphery. Note that a few cells of the meristem are left over at the outer edge of the stone cells. The radial rows are especially clear towards the left. ×220.

Fig. 8. Later stage showing the differentiation of a group of phloem cells from the meristem. ×220.

Fig. 9. Still later stage showing the differentiation of a vascular bundle consisting of both xylem and phloem. ×220.

Fig. 10. Portion of t.s. of a stem showing the inner or normal and the outer or abnormal ring of vascular tissue. ×37.

PLATE IX

Fig. 11. T.s. of a young root showing the primary xylem plate. Note fungal hyphæ in the cortical cells. ×212.

Fig. 12. T.s. of an old root showing the anomaly. ×6.

Fig. 13. T.s. of the central portion of an old root showing primary xylem and part of secondary xylem and medullary rays. ×190.

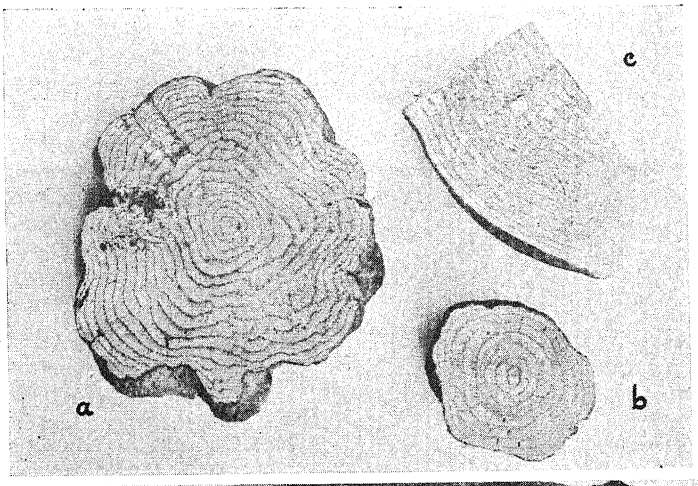

FIG. 2

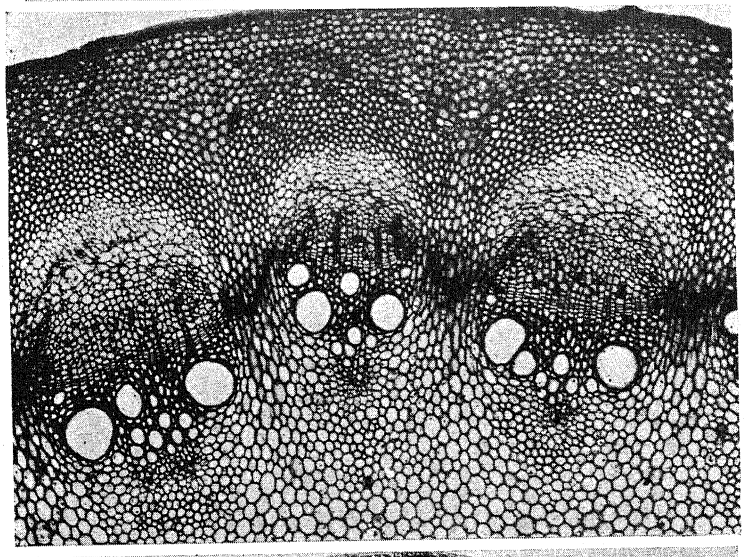

FIG. 3

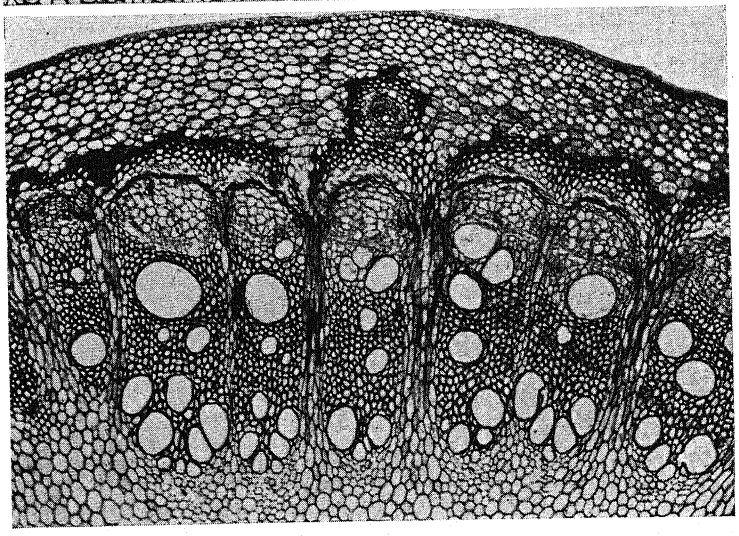

FIG. 4

BALWANT SINGH—
AN ANATOMICAL STUDY OF TILIACORA ACUMINATA *MIERS*

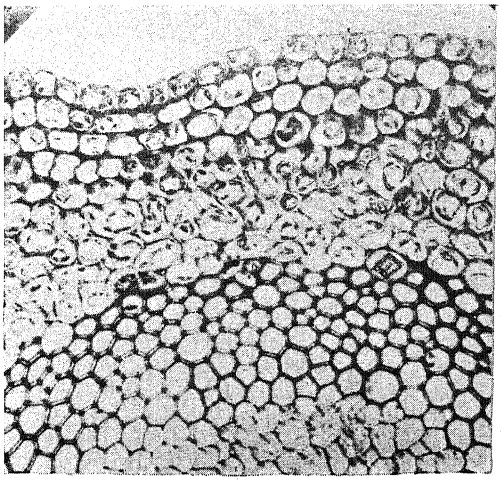

Fig. 5

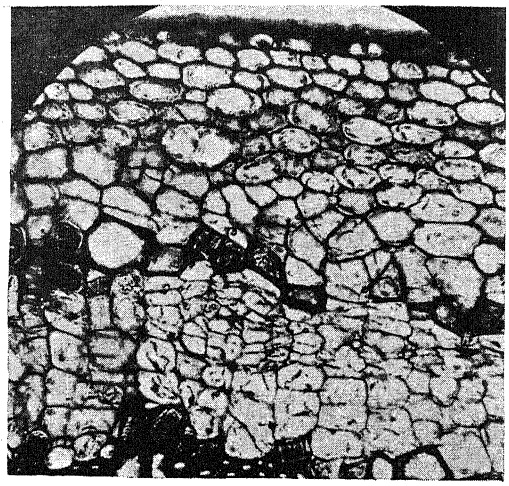

Fig.

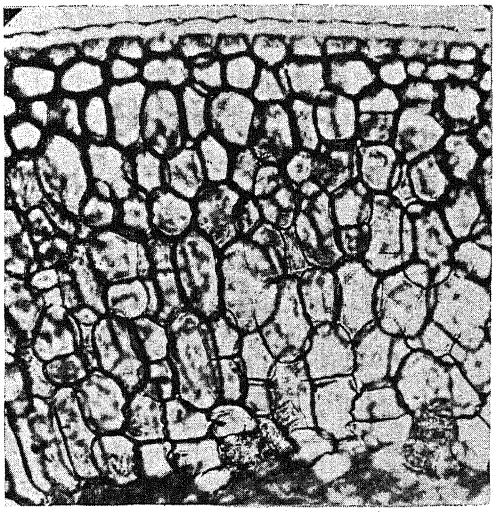

Fig. 6

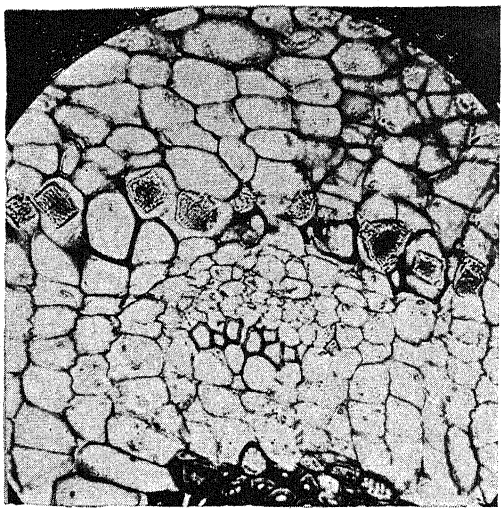

Fig. 9

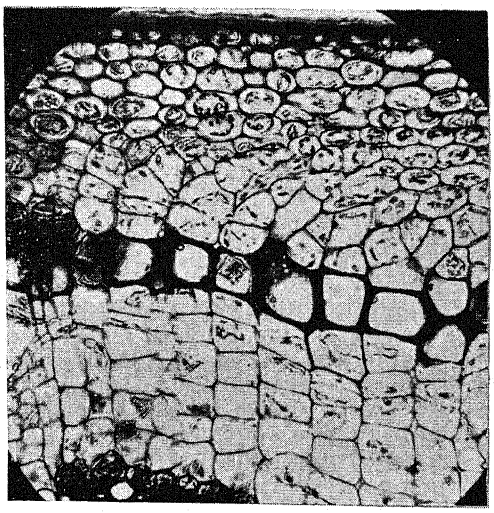

Fig. 7

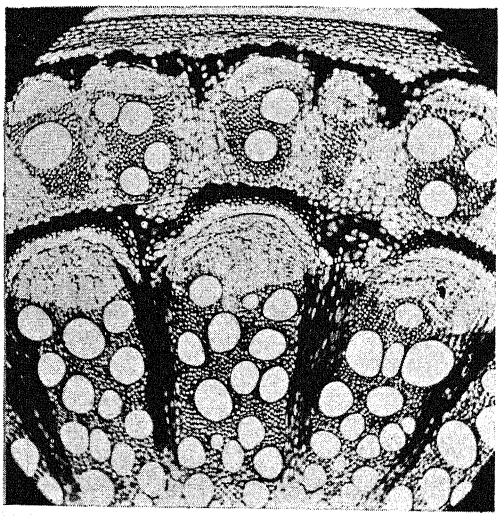

Fig. 10

BALWANT SINGH—

AN ANATOMICAL STUDY OF TILIACORA ACUMINATA *MIERS.*

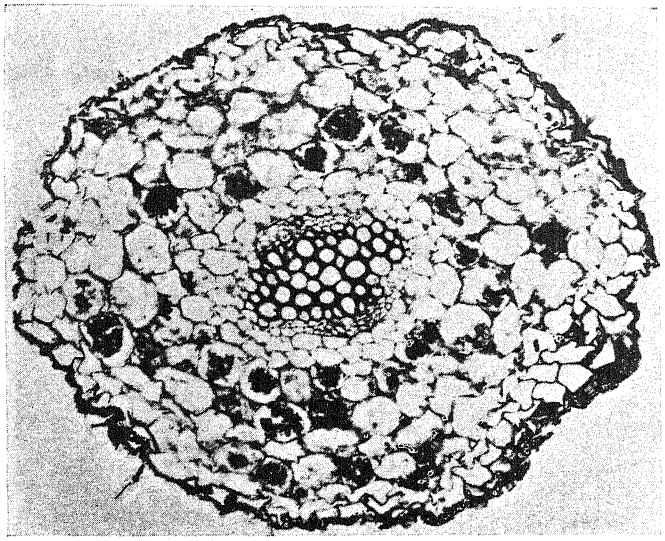

FIG. 11

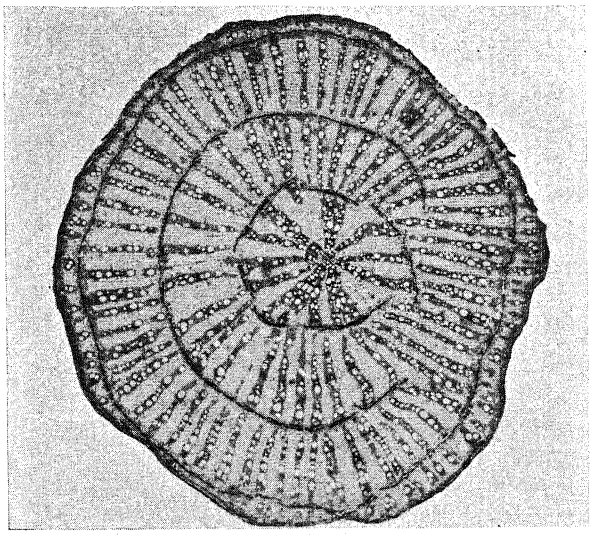

FIG. 12

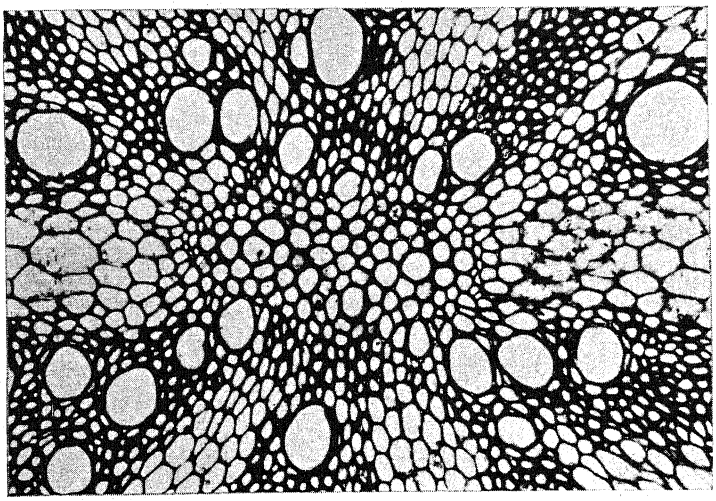

FIG. 13

SOME FOSSIL LEAFLETS OF *AESCULUS INDICA* COLEBR. FROM THE KAREWA BEDS AT LAREDURA AND NINGAL NULLAH, PIR PANJAL, KASHMIR

By G. S. Puri

Department of Botany and Geology, University of Lucknow

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Introduction

This paper is based on a few fossil leaflets collected by the author in 1941 from the Lower Karewa deposits of Kashmir (Lower Pleistocene in age), exposed near Laredura (34° 7′ N.; 74° 21′ E.), a small village at an altitude of 6,000 ft., about 7½ miles from Baramulla. The bridle path leading to Gulmarg at the seventh mile bifurcates into two narrow paths, one of which turns southwest and winds through a thick shrubby growth of *Parrotia jacquemontiana*, *Rosa Webbiana*, *Rubus* spp., etc., in a forest of *Cedrus Deodara* and passes through paddy fields to reach the main huts of the village. The fossiliferous outcrop lies in a steep cliff which is vertically exposed both at top and at the bottom. Our specimens were collected *in situ* from one of the spots lying towards the upper part of the cliff.

The fossil impressions are embedded in a thickly laminated clay, dirty black or yellowish in colour, and splits crudely along the planes of bedding.

This paper has been prepared under the guidance of Prof. B. Sahni, F.R.S., to whom I am profoundly grateful for helpful criticism and ready help. To the Vice-Chancellor, University of the Panjab, and Principal Jodh Singh of the Khalsa College, Amritsar, I am thankful for a research scholarship from the University and also to the authorities of the Lucknow University for award of a Research Fellowship. I take this opportunity to thank Thakur Harnam Singh, D.F.O. of the Kashmir Forest Service, and Messrs. John Clowsley, and F. Xavier of the St. Joseph College, Baramulla, for helping me in making collections from Laredura.

Description

Order : Sapindales

Family : Sapindaceæ

The family is represented in the Karewa Flora by a few leaflet fragments, which are referred to a single species of the genus *Aesculus;*

all the specimens are well preserved impressions. Living plants of this species have compound leaves with 5–7 leaflets arising from a common stalk in a palmate manner. The two lower and outer leaflets are the smallest in size and the one in the middle is the largest. Fossil leaflets of all sizes have been found, but they are all detached and fragmentary specimens.

Aesculus indica Colebr.

Figs. 1–5

The specimens described below are two leaflet fragments, one of which (Fig. 1) is almost complete and measures 4·5 inches long by 2·2 inches in the broadest part, which lies a little above the middle. The lamina, which is oblong-lanceolate in shape, gradually narrows down from the middle into cuneate base. It is broadly acute or short acuminate at the apex. The margins are closely sharp serrate; teeth are small and sharply pointed. Fig. 3 is a natural size photograph of another fragment which, though badly preserved as regards details of venation, illustrates a smaller leaflet. The two leaflets figured here show a good deal of difference in size.

The venation is pinnate and reticulate with a tendency to form small loops under and close to the margins. A strong midrib arises from the base and runs in the lamina slightly thinning out in the upper part. 11–12 secondaries which are almost half as thick as the midirb, arise from the latter on either side in an alternate manner at open angles. The laterals in Fig. 1 curve upwards and inwards to from small shallow loops a little beneath and close to the margins. The loops are not well preserved and do not show up conspicuously even in living leaflets (Figs. 4, 5). The tertiary ribs are small and thin; they arise from the two opposite laterals and run in the area enclosed by them ; and anastomose variously to form large meshes of different shapes and sizes, seen clearly in Fig. 2, which represents a part of a leaflet shown in Fig. 1 enlarged to five diameters. There is a finer reticulation, which consists of a network of small, rectangular or polygonal meshes (Fig. 2).

The fossil leaflets are identical in all respects with modern leaflets of *Aesculus indica* Colebr. (Figs. 4, 5).

Number of Specimens.—Five.

Occurrence.—Spot No. 2 and Spot No. 5 Laredura at 6,000 ft., and Ningal Nullah at 9,000 ft., and locality No. 1, Ningal Nullah at 9,500 ft., Pir Panjal, Kashmir.

Collection.—G. S. Puri, 1941.

Registered Nos. of figured Specimens.—Pl. X, Figs. 1, 2 = L 662/2; Pl. X, Fig 3 = L 814/5.

MODERN DISTRIBUTION OF THE SPECIES

The genus *Aesculus* is included by Hooker in the Sapindaceæ in his *Flora of British India*, but Rendle describes it under the family

Hippocastanaceæ, which contains 2 genera and 18 species, mainly represented at the present time in the United States of America, although they are distributed throughout the north temperate zone.

A very large number of modern species of *Aesculus* occur in North America ; it may be interesting to point out that the species growing on the Atlantic and Pacific sides of the continent are quite different ; one species extends northwards into Canada. In Europe, we have only one species (*A. Hippocastanum*), which is a wild tree of the Albanian and Northern Greece mountains. The Old World is not rich in horse-chestnuts; Japan having two species, while there is only one in Northern China ; and we have two in the Himalayas. The Himalayan species are large handsome deciduous trees, one of which *A. indica* has been found in a fossil state in the Karewas and its modern distribution in India is given below :

The Indian horse-chestnut grows. in the Western Himalayas extending westwards from Nepal at an altitude of 4,000–9,000 ft.; it usually grows in moist shady ravines and prefers northerly aspects of hills, which are comparatively cooler. It is often gregarious along moist gorges and grows plentifully in steep ravines and on hill-sides. In Hazara it is fairly common, occurring with *Juglans regia*, *Acer* spp., *Prunus* spp. and other broad-leaved trees together with coniferous species, namely, *Abies Webbiana*, *Taxus baccata*, *Picea* sp., *Cedrus Deodora*, and *Pinus excelsa*.

The species is fairly common in Kashmir and occurs within a radius of one quarter of a mile of the fossiliferous locality ; it is represented both on the Himalayan slopes and the northern slopes of the Pir Panjal Range at Gurez, Jhelum Valley, Kamraj, Keran, Kishtwar, Marwa Dachhan, Muzaffarabad, Ramban, Sindh Valley and Udhampur. In the regions adjoining Kashmir the species occurs in Kafirstan at 7,000–8,000 ft., Chitral at 7,500 ft., Kagan Valley at 9,000 ft. ; and in the Murree Hills it is associated with *Quercus dilatata*, *Q. incana*, *Prunus* sp., *Acer* sp., *Pinus excelsa*, *Taxus baccata*, etc. Eastwards, it extends to the Parbatti Valley (Kulu at 7,500 ft.), Chamba State, Kangra, Simla Hills, Mussoorie, Tehri Garhwal, Kumaon, Naini-Tal and Almora.

In the "western oak-fir forests of Garhwal Himalayas"*Aesculus indica* is associated with *Quercus semecarpifolia*, *Q. dilatata*, *Ulmus Wallichiana*, *Acer Cæcium*, *Corylus colurna*, *Rosa macrophylla*, *Syringa Emodi*, *Viburnum* spp., *Taxus baccata*, *Abies Pindrow*, *Picea Morinda*, etc. (Champion, 1936, p. 245).

It also occurs according to Champion (*loc. cit.*, pp. 257–58) in the moist temperate forests, *e.g.*, in Dwali (W. Almora division, Kumaon, United Provinces) its associates are *Acer Cæcium. A. pictum*, *Carpinus viminea*, *Ulmus Wallichiana*, *Betula alnoides*, *Juglans regia*, *Fraxinus micrantha*, *Quercus semecarpifolia*, *Corylus colurna*, *Cornus macrophylla*, *Rhus punjabensis*, *Taxus baccata*, *Berberis* spp., *Rubus repens*, etc., but it is associated with *Pinus excelsa*, *Prunus padus*, *Viburnum fœtens*, *Ulmus Wallichiana*, *Acer Cæcium*, *Juglans regia*, etc., in the northwest Himalayas at Saran (Kagan division, Hazara).

Discussion

The Indian horse-chestnut occurs in the Western Himalayas at the present time in two different floristic formations, namely (i) in the Kashmir Valley it occurs with *Juglans regia*, *Rhamnus purpurea*, *Populus ciliata*, *Salix Wallichiana*, *Pinus excelsa*, *Abies Webbiana*, etc., and (ii) at another place it is usually associated with conifers and with oaks and other broad-leaved species characteristic of the Western Himalayan rain forests.

From the available evidence concerning the Karewa flora it seems that *Aeculus indica* during the Early Pleistocene existed with oaks, elms, *Betula*, etc., at one place (Laredura) while its common associates at another place (Ningal Nullah) were willows, cherries, poplars, walnut, etc., etc.

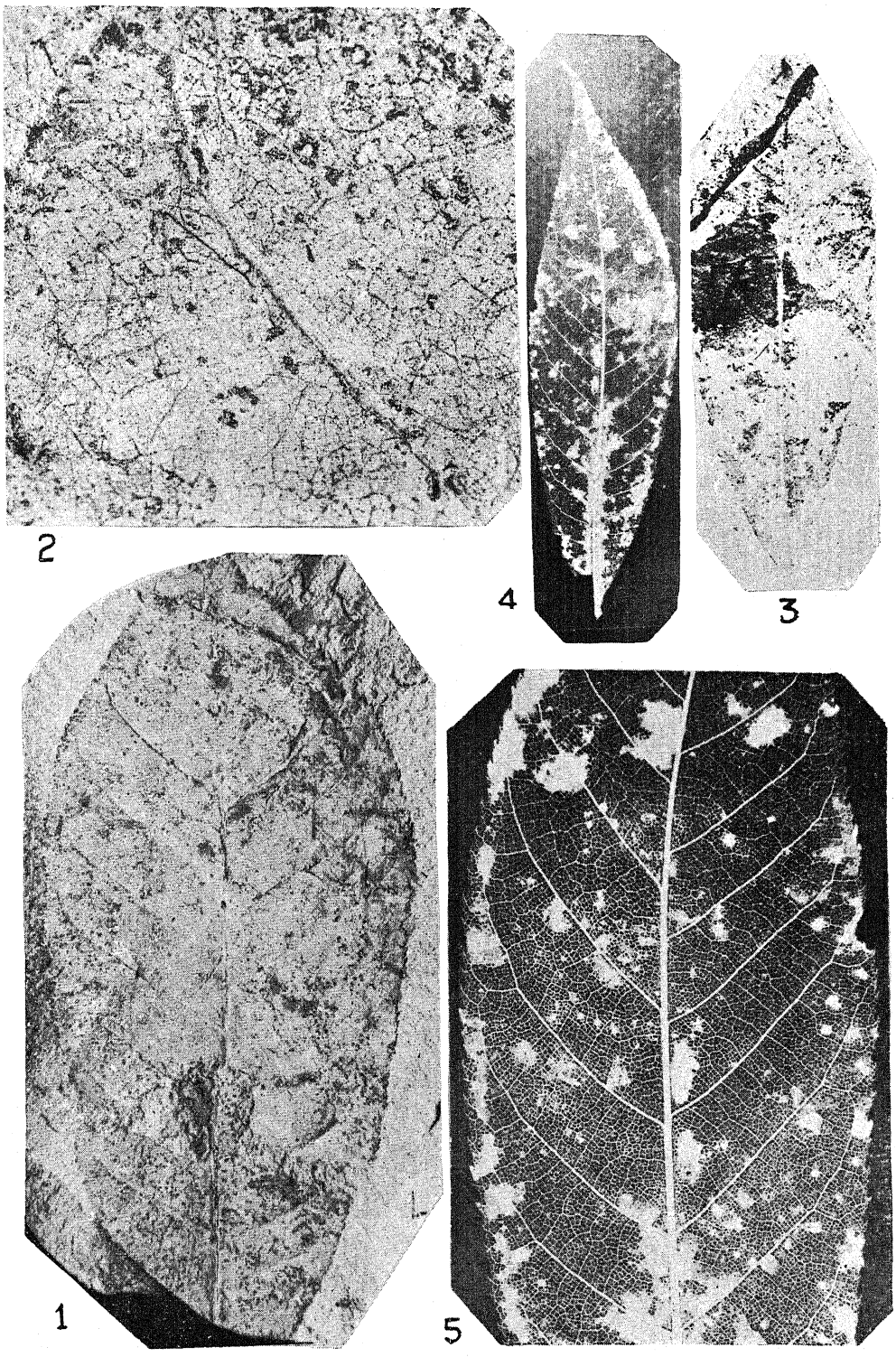
According to Troup (1921, Vol. I, p. 227) the climatic conditions which are most congenial for the growth of *Aesculus indica* under natural habitats include a rainfall varying from 40–100 inches or more ; an absolute maximum shade temperatures between 80° and 102° F. and an absolute minimum varying from 25°–10° F. From this it may be conjectured that the Kashmir Valley during the Early Pleistocene may have had at Laredura and Ningal Nullah climatic conditions somewhat corresponding to the above.

Summary

1. Some leaflets of *Aesculus indica* Colebr. collected by the author from the Lower Karewa deposits (Pleistocene), at Laredura (alt. 6,000 ft.) and Ningal Nullah (alt. 9,000 ft.) are described in detail.

2. The modern distribution of the genus, and of the fossil species is given with special reference to India. At the present time it grows in the Kashmir Valley quite close to the fossiliferous localities and also occurs plentifully at several places in the Kashmir Himalayas and on the Valley slopes of the Pir Panjal Range ; also in the regions adjoining the Kashmir and Jammu territories.

3. The fossil associates of *Aesculus indica* at Laredura and Ningal Nullah are quite different ; and it is pointed out that the Ningal Nullah associates of the species are still existing in the Kashmir Valley, whereas most of the plants, *e.g.*, oaks, *Mallotus*, *Woodfordia*, etc., which were associated with the Indian horse-chestnut at Laredura during the Pleistocene, have now disappeared from the valley proper and the northern slopes of the Pir Panjal Range ; however, the latter occur on the Punjab slopes of the Pir Panjal.



G. S. PURI—

SOME FOSSIL LEAFLETS OF AESCULUS INDICA *COLEBR. FROM THE KAREWA BEDS AT LAREDURA AND NINGAL NULLAH, PIR PANJAL, KASHMIR*

LITERATURE CITED

Champion, H. G. (1936) .. "A preliminary survey of the forest types of India and Burma," *Ind. For. Rec., Silviculture*, **1** (1).

Puri, G. S. (1942) .. "Palæobotany in India, Progress Report for 1941," *Jour. Ind. Bot. Soc.*, **21**, 222–23.

Troup, R. S. (1921) .. *The Silviculture of Indian Trees*, Vol. I, Oxford University Press.

EXPLANATION OF PLATE

All photographs are from untouched negatives. Figured specimens come from G. S. Puri's collection and are preserved in the Botany Museum, University of Lucknow.

Aesculus indica Colebr.

Fig. 1. Fossil leaflet impression. Laredura L 662/3. Nat. size.

Fig. 2. A part of the leaflet enlarged to show meshes of tertiary and finer reticulation. $\times Ca.$ 5.

Fig. 3. Fossil leaflet impression. Laredura L 814/5. Nat. size.

Fig. 4. Modern leaflet, partially rotted before photographing to show venation for comparison with the fossil leaflet (Fig. 4).

Fig. 5. A part of another modern leaflet to show comparison with the fossil leaflet shown in Fig. 1.

STUDIES ON THE EFFECT OF 'SHORT' AND 'LONG DAY' TREATMENT ON THE GROWTH PERIOD AND THE FLOWERING DATES OF DIFFERENT PADDY VARIETIES

BY A. B. SARAN

Bihar Agricultural College, Sabour

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INTRODUCTION

DIFFICULTIES have often been experienced by plant-breeders in effecting crosses between crop varieties differing widely in their flowering dates. This, however, gets more pronounced in a crop like paddy, where the flowering dates differ widely and are more or less fixed for individual varieties or strains, *i.e.*, an early 'Aman' variety shall always flower, under Bihar conditions, in early October whereas the late ones do so at the end of the same month or in early November, when the earlier ones have finished all their flowering and are practically ripe. In such cases, a cross is ordinarily not possible except by utilizing the late tillers of the early variety. This method, however, has its own limitations, specially when the interval between the flowering dates of the varieties to be intercrossed is fairly long. Besides this, the setting in late tillers is usually poor and as such the crosses made at this stage are not always successful. In view of these facts, it was thought that all these difficulties may be successfully solved, if the flowering dates could be suitably altered by increasing or decreasing the 'day length' artificially. This method of altering the time of flowering was initiated by Garner,[4,5,6] who introduced the term photoperiodism to designate the response of plants to the relative length of day and night. Since then many investigators have published results of studies of this phenomenon as it applies to plants. Names of Adams,[1] Evans,[3] Harrington,[7] Ramaley,[8] Shirley,[9] and Tincker[10] may be mentioned as chief workers in the line. Chien-Liang Pan[2] from China published some preliminary results on rice—a crop which does not seem to have been extensively studied and practically no work appears to have been done under Indian conditions. In view of these facts and specially the difficulties encountered by the breeders enumerated above, experiments were taken up to investigate the problem of suitably altering the flowering dates of paddy varieties under Sabour conditions. The results obtained so far are briefly indicated in the present paper.

A. EXPOSING PLANTS TO LONG DAY LENGTH

1. *Material and Method*

In the experiment 30-days old seedlings from one pure strain, 46 BK, were transplanted singly in pots, containing well mixed rice field soil. After a lapse of 30 days from transplanting—*i.e.*, when the

seedlings had fully established themselves—pots showing fairly uniform growth were selected for experimentation. For each treatment there were six plants.

Constant illumination was given by exposing the plants arranged in a ring to a 200 C.P. Petromax light which was kept in centre uniformly lighted throughout the period of darkness, *i.e.*, from sunset to sunrise. The distance from the Petromax to the plants was 2½ feet.

Plants were exposed to constant light for various periods and at different stages of the growth fully indicated in Table I. After the completion of the treatment plants received only sun light and their subsequent performance was watched carefully and their general growth along with their flowering dates were noted and the results obtained are are given in Table I.

TABLE I

Statement showing the effect of constant illumination on growth and time of flowering of a timely 'fixed' paddy variety

Period of constant illumination	Date on which the plants came to flower	Remarks
1	2	3
Set A—		
1. 40 days .. .. (Sept. 1–Oct. 10)	Dec. 12	Growth very poor; flowering delayed by 46 days
2. 30 days .. .. (Sept. 11–Oct. 10)	Nov. 17–18	Growth poor; flowering delayed by about 21 days
3. 20 days .. .. (Sept. 21–Oct. 10)	Nov. 7–9	Growth fair; flowering delayed by about 11 days
4. 10 days .. .. (Oct. 1–10)	Oct. 25–27	Growth practically normal; no change in the flowering date
Set B—		
1. 10 days .. .. (Oct. 1–10)	Oct. 26–27	Growth practically normal; no change in the flowering dates
2. 20 days .. .. (Aug. 22–Sept. 1)	Nov. 6–9	Growth fair; flowering delayed by about 10 days
3. 30 days .. .. (Aug. 22–Sept. 20)	Nov. 16–17	Growth fair; flowering delayed by about 20 days
4. 40 days .. .. (Aug. 22–Sept. 30)	Dec. 8–10	Growth very poor; flowering delayed by about 42 days
5. In constant light from Sept. 1	..	Plants presented a withered appearance. Treatment was therefore discontinued on Nov. 26
6. Received only the usual sunlight (control)	Oct. 26–27	Normal flowering

2. *Results and their Discussions*

From the above table it is clear that by suitably increasing the length of day, time of flowering can considerably be delayed, in so much so that the plants which received constant illumination continuously for a period extending over 30 days beyond the normal flowering, did not come to flower and ultimately the treatment was abandoned as the plants presented a withered appearance. The above results also show that 10 days of constant illumination does not produce any effect on the flowering dates of paddy whereas a period of 20, 30 and 40 days of constant illumination, irrespective of the stage, earlier (set A) or later (set B) in the growth period, at which it is given, bring about a delay in the flowering dates to the extent of 10, 20 and 45 days respectively, *vide* column 3 of Table I.

After establishing the possibility of delaying the flowering time of paddy by subjecting the plants to increased 'day length', experiments were taken up to study in detail the effect of short 'day length' on flowering time of the various classes of paddy varieties.

B. Exposing Plants to Short Day Length

1. *Material and Method*

The material used in this study consisted of five pure varieties of paddies widely differing in their growth periods, when grown under normal conditions, as indicated below :—

(*a*) *Periodly fixed paddies*, i.e., '*Aus*'.—

(i) 'Sathis' takes about 60 days to come to flower from the date of germination, irrespective of the season.

(ii) 'Aus' No. 28–16–21 : takes about 80 days to come to flower from the date of germination, irrespective of the season.

(*b*) *Timely fixed paddies*, i.e., '*Amans*'.—

(iii) Early 'Aman' (115 BK) : flowers between 8–10 Oct.

(iv) Medium 'Aman' (16 BK) : flowers between 20–22 Oct.

(v) Late 'Aman' (36 BK) : flowers between 28–30 Oct.

Three seeds of each of these five varieties were sown on 27th May in pots containing well mixed rice field soil. Ultimately in each pot seedlings were thin down to one. Seedlings of the following age of the varieties under experimentation, as indicated below, were included in the test. Age of the seedlings was counted from the date they emerged out of the soil.

Paddy varieties	Age of the seedlings in days
'Sathi' ..	7, 15 and 30.
'Aus' 28–16–21 ..	7, 15, 30 and 45.
Early 'Aman' ..	7, 15, 30, 45, 60, 75, 90, 105 and 120.
Medium 'Aman' ..	7, 15, 30, 45, 60, 75, 90, 105 and 120.
Late 'Aman' ..	7, 15, 30, 45, 60, 75, 90, 105 and 120.

The 'short days' were given in each case by removing the plants from daylight to a well ventilated dark room at 3 p.m. and 5 p.m. each day. In the following morning at 5 a.m. they were taken out of the dark room and placed outside in the open along with the control plants. Each set was replicated 4 times.

In the following order, the seedlings were subjected to the 'short days' on the dates noted against each of them in Table II.

TABLE II

Paddy varieties	Age of the seedlings in days	Dates on which 'short day' treatment was started
1	2	3
1. 'Sathi', 'Aus' and 'Aman' ..	7	8th June
2. 'Sathi', 'Aus' and 'Aman' ..	15	15th June
3. 'Sathi', 'Aus' and 'Aman' ..	30	30th June
4. 'Aus' and 'Aman'	45	15th July
5. 'Aman'	60	30th July
6. 'Aman'	75	14th August
7. 'Aman'	90	29th August
8. 'Aman'	105	13th Sept.
9. 'Aman'	120	28th Sept.

When the plants came to flower the 'short day' treatment was discontinued in each case and the plants were thenceforth kept in open for further growth along with the controls. Results obtained are fully detailed in Table III.

2. *Results and their Discussions*

It may be seen from Table III that the plants of all the varieties under experimentation in both 3 p.m. and 5 p.m. sets up to 30 days-old seedlings, came to flower at about the same time, end of July, *i.e.*, within 60–63 days of germination. The actual period of earliness in flowering induced by the 'short day' treatment being dependent on the normal flowering dates of these varieties (columns 7 and 8, Table III) is thus different in the different varieties. Consequently the 'Aus' variety flowered 19–20 days earlier than the control whereas the early, medium and late 'Aman' varieties flowered earlier by 67–69, 79–81 and 87–89 days than their respective controls.

The 'Sathi' variety did not at all respond to the treatment. Its 60 days normal period of growth could not be reduced and it thus flowered with the control.

All the varieties in the 45 days set came to flower together on 15th–22nd of August, *i.e.*, about a fortnight later than that of 7, 15 and 30 days sets. In the remaining sets, *i.e.*, 60, 75, 90 and 105, all the varieties under trial flowered together on 1-5th September, 15-18th September, 30th September to 5th October and 14th October to 20th October respectively, *i.e.*,

at fortnightly intervals throughout their growth period. The early paddy in the 105 days set and the medium and late in the 120 days sets, which were near their normal maturity when the light treatment was commenced, did not respond to the treatment and came to flower with the control. The behaviours of the 3 p.m. and 5 p.m. sets, in all these cases as well were almost similar. The induced period of early flowering in these sets as the light treatment was given at later dates (column 3, Table III) was correspondingly shorter as fully detailed in columns 7 and 8 of the table under reference.

It may further be seen from Table III that the seedlings of the age of 30 days and over have taken 30–33 days of the light treatment in both the sets of 3 p.m. and 5 p.m. to come to flower earlier than their controls. But the case of 7 and 15 days old seedlings sets is different in this respect. They have, as may be seen, columns 3 and 4 of Table III, taken longer periods of 'short-days', *i.e.*, 53–55 and 45–47 days respectively in both the sets (3 p.m. and 5 p.m.). When we compare these results with that of the seedlings of older age (30 days and over) it seems highly probable that the extra period of 23–25 days in case of 7 days and 15–17 days in case of 15 days old seedlings have been utilised in vegetative growth to make a total of 30 days and the net period of 'short-days' to induce early flowering is probably only 30–33 days as is the case with older seedlings, *i.e.*, 30 days and over.

It is interesting to note from these studies that a period of 60 days is the very minimum for any variety of paddy to come to flower. The 'Sathi' having 60 days growth period are thus the earliest paddy in nature.

These studies have opened out a new field for hybridization work in paddy by enabling us not only to make paddy varieties widely differing in their normal flowering dates to flower simultaneously, any time in the season, but also to make individual plants of any particular variety to come to flower, one after the other, throughout its growing period—as against hardly for a fortnight at fixed time—and thus get ample material and opportunity for intercrossing it with large number of other varieties and strains with different periods of maturity and flowering dates.

It may incidentally be pointed out that the late variety, which was under study in pots, was also grown in four small beds in one of the paddy fields. When the seedlings were 30 days old, two of them were covered with moveable Tati-covers at 3 p.m. each day. After about 32 days of this treatment, the two experimental beds came to flower. Thus by this light treatment any individual plant or a set of plants in a plot can be made to flower at any suitable earlier date and thus serve a variety of purpose.

It may finally be mentioned that quite a good amount of data have been collected on the growth and yield of the plants subjected to these light treatments, which will form the subject-matter for next paper. But it will not be out of place to mention in a general way that the height of the plants subjected to 'short day' treatment gets increased.

TABLE III

Table showing the effect of shortening the 'day length' at different stages of growth on the flowering dates of different paddy varieties

Age of seedlings before receiving the treatment	Paddy variety	Total number in days for which plants received 'short days' before coming to flower. Figures in brackets show the dates on which the treatment was started in both 3 p.m. and 5 p.m. sets		Date of flowering		Average period in days of induced early flowering as compared to the control	
				Hours for which plants received sunlight			
		3 p.m. set	5 p.m. set	3 p.m. set	5 p.m. set	3 p.m. set	5 p.m. set
1	2	3	4	5	6	7	8
		(8th June)					
7 days' old ..	'Sathi'	53	53	31st July–1st Aug.	31st July–1st Aug.	Flowered	with control
	'Aus' (28–16–21) Early (115 BK)	54	53	1st–2nd Aug.	31st July–2nd Aug.	19	20
	'Aman' Medium (16 BK)	53	55	31st July –1st Aug.	2nd–3rd Aug.	69	67
	Late (36 BK)	55	53	2nd–4th Aug.	31st July–2nd Aug.	79	81
		53	55	31st July–2nd Aug.	2nd–4th Aug.	89	87
		(15th June)					
15 days' old ..	'Sathi'	47	45	2nd–3rd Aug.	31st July–2nd Aug.	Flowered	with control
	'Aus' (28–16–21) Early	45	46	31st July–2nd Aug.	1st–2nd Aug.	20	19
	'Aman' Medium Do.	47	45	2nd–4th Aug.	1st–2nd Aug.	67	68
	Late	45	45	31st July–3rd Aug.	31st July–3rd Aug.	81	81
		46	47	1st–3rd Aug.	2nd–3rd Aug.	88	87
		(30th June)					
30 days' old ..	'Sathi'	31	30	31st July–1st Aug.	30th–31st July	Flowered	with control
	'Aus' (28–16–21) Early	29	30	29th July–1st Aug.	30th July–2nd Aug.	22	21
	'Aman' Medium Do.	32	30	1st–4th Aug.	30th July–3rd Aug.	68	69
	Late	29	30	29th July–2nd Aug.	30th July–4th Aug.	73	82
		32	30	1st–4th Aug.	30th July–3rd Aug.	88	90

45 days' old ..	'Aus' (28–16–21)	(15th July)					
	Early	32	30	17th–20th Aug.	15th–18th Aug.	3	5
	'Aman' Medium Do.	32	30	17th–20th Aug.	15th–18th Aug.	52	54
	Late	35	33	20th–22nd Aug.	18th–21st Aug.	61	63
		36	34	21st–22nd Aug.	19th–22nd Aug.	68	70
60 days' old ..		(30th Aug.)					
	Early	32	33	1st–3rd Sept.	2nd–3rd Sept.	37	36
	'Aman' Medium Do.	33	32	2nd–4th Sept.	1st–4th Sept.	48	49
	Late	32	33	1st–4th Sept.	2nd–5th Sept.	57	56
75 days' old ..		(14th Aug.)					
	Early	31	32	15th–18th Sept.	16th–18th Sept.	23	22
	'Aman' Medium Do.	32	31	16th–17th Sept.	15th–17th Sept.	34	35
	Late	32	31	16th–18th Sept.	15th–17th Sept.	42	43
90 days' old ..		(29th Aug.)					
	Early	32	32	1st–4th Oct.	1st–3rd Oct.	7	7
	'Aman' Medium Do.	31	32	30th Sept.–2nd Oct.	1st–2nd Oct.	20	19
	Late	33	31	2nd–5th Oct.	30th Sept.–4th Oct.	26	28
105 days' old		(13th Sept.)					
	Early	23	22	8th–9th Oct.	7th–8th Oct.	Flowered with control	
	'Aman' Medium Do.	30	29	15th–16th Oct.	14th–16th Oct.	5	6
	Late	32	31	17th–20th Oct.	16th–20th Oct.	11	12
120 days' old ..		(28th Sept.)					
	Early	..	..	..	..	..	..
	'Aman' Medium Do.	18	20	18th–20th Oct.	20th–21st Oct.	Flowered with control	
	Late	29	28	29th–30th Oct.	28th–30th Oct.	Flowered with control	

'Sathi'	31st July–1st Aug.	
'Aus' (28–16–21)	20th–22nd Aug.	Normal flowering date
Early	8th–10th Oct.	
'Aman' Medium	20th–22nd Oct.	
Late	28th–30th Oct.	

Number of tillers, dry weight of straw and yield per plant does not seem to differ very much from that of the control plants.

Lastly, it may be mentioned that other aspects of the problem, such as the effect of 'short day' treatment, given at the seedling stage on the growth and flowering of the transplanted crop and other inter-related questions are under investigation and the results will be presented later.

Summary and Conclusions

A. 'Long day' treatment.

1. By increasing the 'day lengths', *i.e.*, by exposing the plants to artificial light during the night, flowering time of paddy can be suitably delayed—the actual shifting of the flowering dates depends on the duration of the 'long day' treatment given.

B. 'Short day' treatment.

1. Any variety of paddy, irrespective of the class or maturity period to which it may belong, can be induced to flower within 60–63 days of germination.

2. With 'short day' treatment, different paddy varieties with different flowering dates can not only be made to flower simultaneously but individual plants of any particular variety can also be induced to flower, one after another throughout the growing period—thus offering ample material and opportunity for intercrossing it with a number of other varieties with different maturity periods.

3. With this treatment, even 'Aus' varieties, whose period of growth between sowing and flowering is fixed and rather short, can be induced to flower earlier.

4. The 'Sathi' variety did not at all respond to the treatment. Its 60 days normal period of growth could not thus be reduced any further.

5. 'Short day' treatment method has been found to be easier to work with than the 'long day' one specially when Petromax is used as a source of light.

Acknowledgment

Grateful acknowledgment is due to late Mr. M. Alam, M.Sc., F.L.S., Rice Specialist, for his suggestions and advice during the conduct of these experiments and to Dr. R. H. Richharia, M.Sc., Ph.D. (Cantab.), Economic Botanist, Bihar, Sabour, for taking keen interest in the work.

LITERATURE CITED

1. Adams, J. (1924) .. "Duration of light and growth," *Ann. Bot.*, **38**, 509–23.
2. Chien-Liang Pan (1936) "Length of exposure to light in relation to plant growth in rice," *Jour. Amer. Soc. Agronomy*, **28**, No. 1, 58–63.
3. Evans, M. W. (1931) .. "Relation of latitude to time of blooming of timothy," *Ecology*, **12**, 182–87.
4. Garner, W. W. (1920) "Flowering and fruiting of the plants as controlled by the length of day," *U. S. D. A. Year Book*, **1920**, 377–400.
5. ——— and Allard, H. A. (1920) "Effect of the relative length of day and night and other factors of environment on growth and reproduction in plants," *Jour. Agr. Res.*, **18**, 553–606.
6. ———— (1931) .. "Effect of abnormally long and short alterations of light and darkness on growth and development of plants," *ibid.*, **42**, 629–51.
7. Harrington, J. B. (1927) "Growing wheat and barley hybrids in winter by means of artificial light," *Sci. Agr.*, **7**, 125–30.
8. Ramaley, F. (1931) .. "Some Caryophyllaceous plants influenced in growth and structure by artificial illumination supplemental to daylight," *Bot. Gaz.*, **92**, 311–20.
9. Shirley, H. L. (1929) .. "The influence of light intensity and light quality upon the growth of plants," *Amer. Jour. Bot.*, **16**, 354–90.
10. Tincker, M. A. H. (1925) "The effect of length of day upon the growth and reproduction of some economic plants," *Ann. Bot.*, **39**, 721–54.

PROF. S. L. GHOSE

OBITUARY

PROF. S. L. GHOSE

(1893–1945)

A STRANGE fate attends the chair of Botany at Government College, Lahore. The late Professor S. R. Kashyap, who enjoyed robust health and had only the previous year returned from a long trek in the Inner Himalayas and Tibet, died suddenly in 1934, of heart failure, at the early age of 52. Dr. S. L. Ghose, who succeeded him, has now passed away at the same age. Although it was known that he had been keeping indifferent health for some years, none expected that the end would come so soon. The news of his premature death has come as a great shock to his numerous friends, colleagues and pupils.

Dr. S. L. Ghose, born on 13th of December 1893, was the youngest son of Mr. N. C. Ghose, who served for many years in the Education Department of the Punjab and the North-West Frontier Province as Headmaster of several high schools. After a brilliant academic career as a student of the Forman Christian College and the Government College in Lahore, S. L. Ghose took the M.Sc. Degree in Botany of the University of Panjab in 1914, and started life as Demonstrator in Botany in the Allahabad University. He served here for one year. Next year he returned to his *alma mater* as Lecturer in Botany, to work under his own teacher, the late Professor Dr. S. R. Kashyap. He went to Cambridge in 1921 and studied under the guidance of Prof. Seward and Dr. Borradaile. He was awarded the Doctorate in Philosophy in 1923. On his return to India after an extensive tour of continental universities, he was offered the Chairmanship of the newly established Department of Biology at the University of Rangoon. In 1928, however, he returned to the Government College, Lahore. On the death of Dr. Kashyap, he was appointed Professor of Botany. He held this post with distinction until his death on March 24, 1945.

Professor Ghose took keen interest in botanical investigations throughout his life. Some of his earlier papers dealt with the morphology of *Selanginella*, conifers and flowering plants, but his later years were devoted entirely to the study of algology. His investigations on the Myxophyceæ of Northern India and Burma are of an outstanding nature, and by his pioneer work he mapped out for fellow botanists in his country a field hitherto practically unexplored. But more than an investigator, he was a teacher. By his genial temperament and sympathetic approach he had endeared himself to all his pupils. He was always ready to help them even at considerable personal inconvenience, but like a true scientific worker, kept an open mind on the subject under discussion and would never try to enforce his own ideas even on his own pupils.

He was elected Vice-President of the Indian Botanical Society for 1931, 1932 and 1938, and was President of the Society for 1941. He presided over the Botany Section of the Indian Science Congress at Patna in 1933. He was one of the Foundation Fellows of the National Institute of Sciences of India.

By his death, Indian algology loses one of its foremost workers and the University of Panjab an inspiring Professor of Botany.

M. S. RANDHAWA.

REVIEW

Root Disease Fungi. By S. D. Garrett, M.A., D.I.C. Annales Cryptogamici et Phytopathologici, Vol. I, 1944. Waltham, Mass., U.S.A.: The Chronica Botanica Co.; Calcutta: Messrs. Macmillan & Co., Ltd. Pp. 177. $ 4·50.

THIS notable publication, under the new series "Annales Cryptogamici et Phytopathologici," edited by Dr. Frans Verdoorn, is perhaps the first of its kind written on the epidemiology of soil-borne disease in crop plants, and contains certainly the first exposition of the principles of Root Disease control. Mr. Garrett is a leading authority on this widely dispersed group of root-infecting fungi, having given a new orientation to the study of these pathogens in their natural habitat, *the Soil.* From the ecological point of view of these soil organisms, precious little work had been done until the classical work of Waksman, Reinking and others was published early this century. There is no doubt whatsoever to-day that the soil, in general, represents a complex microflora actively competing for the organic and inorganic food material resulting in the inevitable chain of events like antagonism, specialization in food requirements, etc. To a large extent the original hypothesis of Waksman, *viz.*, "that there are in most soils a basic cosmopolitan fungus flora of *soil inhabitants*, among which were to be found exotic fungi or *soil invaders*" has found support from both temperate and tropical workers engaged in this problem. Mr. Garrett's book admirably summarizes this and other allied problems.

The contents of the book under review are arranged under the following chapter headings: (1) Introduction; (2) Parasitic specialization in the root-infecting fungi; (3) Parasitic activity of the root-infecting fungi; (4) Influence of soil temperature upon parasitic activity; (5) Influence of soil moisture content, texture, and reaction upon parasitic activity; (6) Influence of soil organic content and concentration of plant nutrients upon parasitic activity; (7) Saprophytic activity of the root-infecting fungi; (8) Dormancy of the root-infecting fungi; (9), (10) and (11) Control of root disease in field crops: Crop rotation; Plant sanitation; Disease control under the growing crop; (12), 13 and (14) Control of root disease in plantation crops: on virgin areas; in mature plantations and on re-planted areas; special problems; (15) Control of root disease in glasshouse crops. A very exhaustive bibliography terminates the subject-matter of the entire text and is followed by two indices, general and author.

Of special interest to Plant Pathologists are the chapters on "Control of root disease in field and glasshouse crops". Quite a large number of wilts due to fungal attacks are commonly encountered in every-day cultural practices and in the tropics, particularly in seedling beds. Mr. Garrett has focussed sufficient attention in dealing with the subject-matter that forms these chapters. The book on the whole

presents a logical and sequential case into the various fundamental and applied aspects of root-infecting fungi and the author and the publishers deserve congratulation on bringing this volume out. The student of Botany and the Researchers on Soil Fungi in this country will amply benefit by reading this well-written, concise volume which summarises all the latest researches on this important group of soil micro-organisms.

T. S. SADASIVAN.

642-45. Printed at the Bangalore Press, Mysore Road, Bangalore City, by G. Srinivasa Rao, Superintendent.

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GLUCOSE SUCROSE RATIO AND RESPIRATORY DRIFTS IN SUGARCANE

By K. N. Lal and J. S. Srivastava

Plant Physiological Laboratory, College of Agricultural Research, Benares Hindu University

Received for publication on March 24, 1945

Introduction

Studies in juice characters of cane (Singh, 1940) have shown marked variations in sucrose and glucose percentages with advance in age of the crop. A more or less inverse relationship has been noted between these two juice characters inasmuch as with increase in age, sucrose content of juice, in general, rises while glucose content shows a decline (Singh, 1941; Singh and Mathur, 1938). No attempt has so far been made to trace the relation between the respiration rate of successive nodes and glucose sucrose ratio in juice. In the present paper light is thrown on (i) the effect of development of tillers upon sucrose and glucose percentages in juice and upon respiration rate of nodes, and (ii) the possible relationship between the sugar substrate and respiratory intensity in tillers of varying developmental stages.

Method and Material

The experiments were conducted on Co. 312 grown on the Experimental Farm of the College. Towards the end of the adolescent stage (10 months) few healthy bushes were sampled. Tillers of various developmental stages were picked up and arranged in accordance with their increasing height, begining from the youngest on one side and the old well mature tillers on the other. A number of such tiller classes, 1–15, were thus grouped. The entire group contained tillers of varying age ranging between 5–6 months (tiller classes 1–5), 6–7 months (tiller classes 6–9), 7–8 months (tiller classes 10–12) and 8–10 months (tiller classes 13–15). Stripped canes belonging to each of these classes were divided into three equal size pieces and designated as top, middle and bottom canes according to their respective position on the shoot. Green tops were invariably discarded. Each of these portions were

separately crushed and requisite quantity of juice was analysed with respect to sucrose and glucose percentages. Sucrose was estimated by saccharimeter and glucose by Fehling's solution.

Respiration rate of representative nodes (4" in length) from each of the three pieces (top, bottom and middle) and selected from all the different classes of tillers was determined at 31° C. by continuous current method using standard baryta solution as an absorbent. Respiration of the top-most node in the green non-millable cane was also determined. All records have been expressed in milligrammes of carbon dioxide per 100 gm. fresh weight per hour. The cut end of the nodes were invariably sealed with a wax-vaseline mixture and allowed overnight rest before actual respiration measurements were undertaken.

Experimental Results

A. *Sucrose Glucose Percentages and Respiratory Drifts in Top Portion of Cane*

Respiration rate of topmost node situated in the green non-millable cane varied enormously from tiller to tiller (Table I). In the

Table I

Respiration from sugarcane sets from various heights on the cane shoot in different tillers of a bush

(Resp. in mgm. carbon dioxide per 100 gm. fresh weight per hour)

Tiller No.	Position of the sets on the tiller				Tota
	Top-most	Top	Middle	Bottom	
1	170·23	118·30	95·20	66·44	450·19
2	148·87	147·34	82·29	57·47	435·97
3	143·29	117·07	69·27	75·65	405·18
4	126·34	86·21	85·18	62·25	359·45
5	155·04	88·80	60·10	42·01	345·98
6	38·19	35·55	29·23	24·49	127·46
7	28·30	14·66	11·77	11·80	67·53
8	20·41	12·83	7·18	8·78	49·46
9	17·36	16·02	12·05	10·90	56·28
10	22·19	8·86	5·38	3·55	39·04
11	15·32	13·12	8·08	8·78	45·31
12	13·28	10·83	4·05	4·77	32·95
13	27·30	10·76	10·73	10·11	38·90
14	22·78	21·59	10·35	14·90	69·09
15	29·06	11·67	11·01	11·24	61·01

youngest tiller it was highest, gradually decreased with advance in age of the tiller and finally attained a very low level in the oldest cane of the bush. All the younger tillers (5–6 months old) showed a relatively high rate of carbon dioxide output. There was, in general, a continuous slow decline in respiratory activity with increase in age, which attained a very low level in the twelfth tillers (8 months old) of the

bush. Slight rise in activity was again noted in the 8–10 months old canes (Tiller number 13–15), but the increases observed seldom attained the values recorded for the tillers of younger age.

Respiration rate of another node situated at a slightly lower level than that of the above also showed a more or less similar high rate of respiration in younger 5–6 months old tillers and a gradual fall with advance in age till a more or less level course was attained in 8–10 months old canes (Table I, Fig. 1). In almost all tillers the topmost node exhibited a higher rate of respiration than that situated slightly below it. The differences in respiration rate were more discernible in the younger tillers than in the old. High rate of respiration of the top and topmost nodes of 5–6 months old tillers, was apparently due to the high concentration of reducing sugars in these regions, as also the greater protoplasmic activities in the younger regions of tiller number 2–5. The younger the age of the tiller, the higher was the concentration of reducing sugars and in consequence the greater were the rates of respiration.

Sucrose content of the top canes on the contrary, exhibited the reverse course. In the youngest tiller the percentage was low ; it gradually rose to a high value with increase in age of the cane. Tiller number 5–8 showed a level course of sucrose. Further increase in age resulted in still higher values of sucrose (Table II, Fig. 1). Marked

TABLE II

Sucrose percentage of the sets from various heights on the stem of different tillers in a bush

Tiller No.	Position of the sets on the tiller			Total
	Top	Middle	Bottom	
1	8·30	12·30	12·30	32·90
2	10·70	12·80	14·50	39·00
3	13·20	15·05	14·50	42·75
4	14·50	15·70	16·05	46·25
5	13·10	15·10	15·80	44·00
6	13·60	16·40	15·80	45·80
7	13·05	13·10	11·45	37·60
8	13·60	17·70	17·40	48·70
9	17·30	17·30	17·20	51·80
10	16·30	16·90	16·70	49·90
11	15·40	18·20	18·10	51·70
12	16·60	18·20	16·40	50·20
13	14·70	17·30	13·10	45·10
14	19·20	18·00	17·00	54·20
15	17·95	17·25	16·35	51·55

fluctuations were, however, noted in the sucrose content of the entire group of old tillers. These did not coincide with the fall or rise in respiration rate always, although occasionally some such relation was exhibited.

The glucose content, on the other hand, exhibited a course which was more or less reverse to that of the sucrose percentage in the old tillers and a slightly parallel course in the younger 5–6 months old tillers.

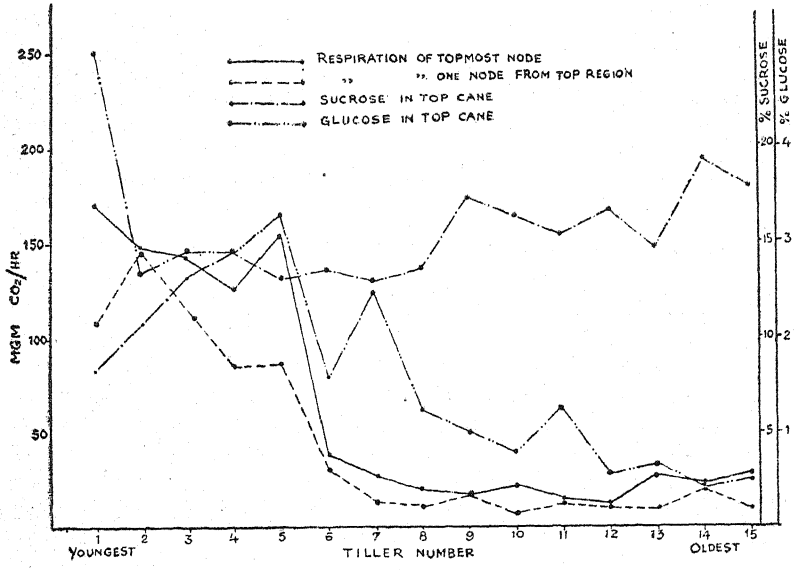


Fig. 1. Respiration, and sucrose and glucose percentages in tillers of various developmental stages. Relative age of tillers: Nos. 1–5 (5–6 months), Nos. 6–9 (6–7 months), Nos. 10–12 (7–8 months), and Nos. 13–15 (8–10 months).

The fall in glucose concentration particularly in the old canes invariably coincided with the rise in the sucrose value and *vice versa* (Table III,

TABLE III

Glucose percentage of the sets from various heights on the stem of different tillers in a bush

Tiller No.	Position of the sets on the tiller			Total
	Top	Middle	Bottom	
1	5·00	4·10	2·20	11·30
2	2·70	2·50	2·50	7·70
3	2·90	2·50	2·00	7·40
4	2·90	2·50	2·50	7·90
5	3·30	2·50	1·80	7·60
6	1·60	1·10	1·40	4·10
7	2·50	2·00	2·50	7·00
8	1·26	1·25	1·00	3·50
9	1·00	0·71	1·00	2·71
10	0·82	1·00	0·71	2·53
11	1·25	1·00	0·71	2·96
12	0·55	0·50	0·15	1·20
13	0·66	0·30	0·50	1·46
14	0·42	0·55	0·91	1·88
15	0·50	0·54	0·71	1·75

and Fig. 1). High glucose and low concentration of sucrose were usually associated with high respiration, and low glucose and high sucrose with low respiration rates.

B. *Sucrose Glucose Percentages and Respiratory Drifts in Middle Portion of Cane*

In the middle portion of different canes the course of respiration of a single node again showed a fall with advance in age of tillers. The fall was sharp in the younger 5–7 months' old tillers, but attained more or less a level course throughout the 7–10 months' old canes (Table I, Fig. 2). Sucrose content, on the contrary, was increasing

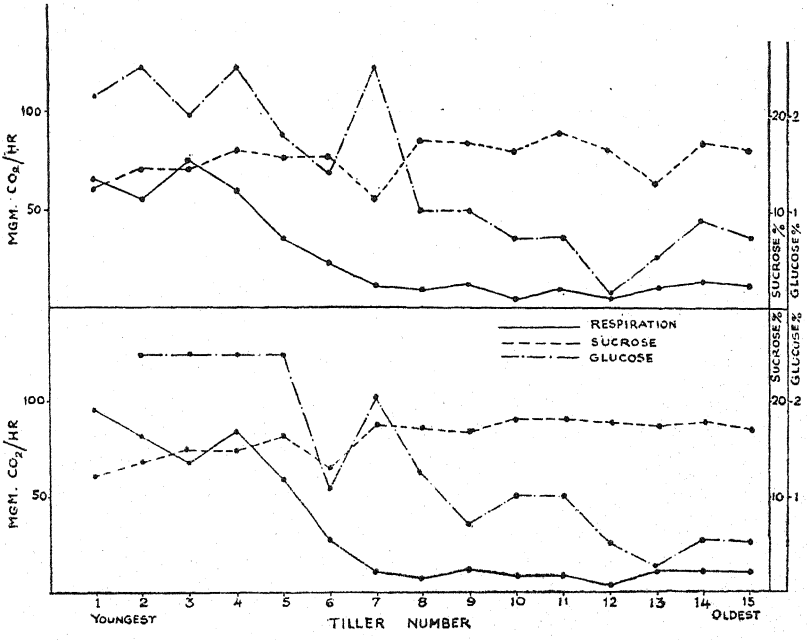


Fig. 2. Respiration, and sucrose and glucose percentages in middle and bottom portion of cane from tillers of different developmental stages—middle (below), bottom (above). Relative age of tillers : Nos. 1–5 (5–6 months), 6–9 (6–7 months), 10–12 (7–8 months), and 13–15 (8–10 months).

with advance in age, attained a high but level phase in the older (8–10 months) tillers and showed only a negative relation with respiration. The concentration of reducing sugars in the younger canes was more or less constant, but fell down as the age advanced. The fall in glucose concentration was more marked in spite of similar level of sucrose in older tillers. Glucose again showed some parallelism with the course of respiration while sucrose indicated only, if at all, a reverse relationship ; this was, however, not as conspicuous as in the top portion of the cane. Here again, high rate of respiration was due to the relatively high concentration of reducing sugars ; the younger the respiring node, the more was the glucose concentration and the higher were the respiration rates (Tables I and III).

C. *Sucrose Glucose Percentages and Respiratory Drifts in Bottom Portion of Cane*

The course of respiration of a node collected from the basal portion of the cane also followed the same general sequence of decline with advance in age of the respective cane. The sucrose content showed a rising tendency, attained high percentage in 8–10 months' old canes and was maximum in the eleventh tiller of the bush (Fig. 2). Subsequent advance in age of the tillers lowered the percentage of sucrose slightly. Glucose showed a general decline with increasing age and reached lowest concentration in the twelfth tiller. Further advance in age caused a rise in glucose concentration ; this was evident in 10 months' old canes of the bush. No relationship was observed between the rise or fall in glucose and sucrose values in the different canes.

D. *Average Respiration Rate and Glucose/Sucrose Ratio*

The average respiration rate of a cane and the average glucose and sucrose percentages are shown in Table IV and Fig. 3. While

TABLE IV

Average respiration and juice characters of various tillers

Tiller No.	Respiration	Sucrose	Glucose	$\frac{\text{Sucrose}}{\text{Glucose}}$	$\frac{\text{Glucose}}{\text{Sucrose}}$
1	112·54	10·96	3·77	2·91	3·44
2	108·99	13·00	2·56	5·08	1·97
3	101·29	14·25	2·46	5·79	1·72
4	89·86	15·08	2·63	5·73	1·74
5	86·49	14·66	2·53	5·79	1·72
6	31·86	15·26	1·36	11·22	0·08
7	16·63	12·53	2·33	5·73	1·86
8	12·36	16·23	1·16	14·00	0·07
9	14·07	17·26	0·90	19·18	0·52
10	9·76	16·63	0·84	19·79	0·05
11	11·33	17·23	0·99	17·40	0·05
12	8·24	16·73	0·40	40·18	0·02
13	9·72	15·03	0·49	30·68	0·03
14	17·27	18·07	0·63	28·69	0·03
15	15·25	17·18	0·58	29·60	0·03

the average rate of respiration exhibited a gradual decline with advance in age of the tiller, the average sucrose content showed an increase. Glucose concentration varied more or less in the same direction as respiration rate though the parallelism was not evidenced sharply at all stages of the development of the tiller. The sucrose/glucose ratio exhibited a low value in the younger,5–7 months' old canes, and a gradual increasing value in the older ones. Glucose/sucrose ratio, on the contrary, indicated high values in the younger tillers and a very low value in 8–10 months' old canes. Respiration showed strictly parallel course with glucose/sucrose ratio except in the case of the seventh tiller (Fig. 3). The younger the age, the more was the rate of

respiration ; this high rate was invariably associated with high concentration of glucose, low sucrose and high glucose/sucrose ratio. Approximately sevenfold increase in respiration rate in the youngest cane was in confirmity with 4–7 times rise in glucose percentage and 6–10 times increase in glucose/sucrose ratio (Table IV).

Variations in respiration rate and sucrose and glucose percentages in tillers of various stages of development point out the importance of respirable substrate on the output of carbon dioxide. Glucose concentration appears to have a controlling influence upon cane respiration, and the ratio that it bears to the sucrose percentage (glucose/sucrose ratio) shows more rigid a relationship to the intensity of

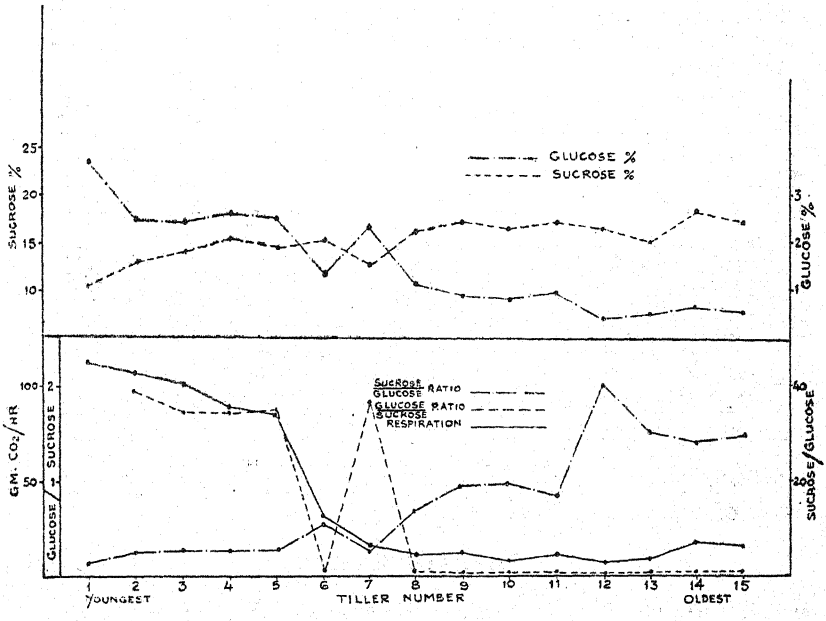


Fig. 3. Average glucose and sucrose percentages in cane juice and sucrose/glucose and glucose/sucrose ratios in relation to respiration rate of canes. Age of tillers : Nos. 1–5 (5–6 months), 6–9 (6–7 months), 10–12 (7–8 months), and 13–15 (8–10 months).

respiration. In younger tillers, intensity of metabolism and rapidity of growth, demand quick supply of easily assimilable sugars and this is amply secured by the high proportion of glucose in comparison to sucrose in young tillers. In old canes, where tissue development has proceeded beyond a certain limit, the demand of the less active tissues on the readily respirable substratum falls down considerably and this explains why there is a preponderance of more stable sucrose over the reducing sugars. The younger tillers are in a way more physiologically active than the older canes in a single bush. In any comparative study of respiration rates of varieties, or for the matter of that, of other treatments, therefore, care needs to be taken to

extend respiration measurements to as many tillers of the bush as practicable so as to get a representative picture of the nature of response.

SUMMARY

The paper deals with the respiratory drifts in canes of various stages of development selected from a field crop of sugarcane var. Co. 312. Height of the shoot was taken as the criterion for the classification of the various groups of tillers. Fifteen groups were sampled and arranged in order of increasing height; these included canes 5–6 months' old (tiller number 1–5), 6–7 months (tiller number 6–9), 7–8 months' (tiller number 10–12), and 8–10 months' old cane (tiller number 13–15). The millable portion of these stripped canes only were selected and equally divided into three parts—top, middle and bottom.

Respiration rate of a single node in sets four inches long and collected from (i) extreme apex in the green non-millable cane, (ii) top region, (iii) middle portion and (iv) bottom piece of each of the above tillers was measured. Sucrose and glucose concentrations in extracted juice from top, middle and bottom pieces were separately estimated.

Irrespective of the portion from which a set was selected for measurement of respiration rate, the intensity of carbon-dioxide output declined with each successive increase in age of tillers. Younger tillers were physiologically more active than the old and mature canes. In each class of tillers again the respiratory activity was highest at the top and decreased as one proceeded downwards.

Variations in respiration rate were found to be related to fluctuations in glucose more than sucrose, particularly in tillers of the older group. In almost all tillers, glucose/sucrose ratio appeared to determine the intensity of respiration of cane sets. Physiological significance of such changes has been discussed.

LITERATURE CITED

1. Singh, B. N. (1940) .. *Progress Report of the Scheme on the Physiology of Wheat and Cane*, Imperial Council of Agricultural Research, New Delhi, 1938–40.

2. ——— (1941) .. "The Growth of the Sugarcane Plant in India: Part I. Age-fertiliser effects on the physiology and chemistry of sugarcane," *Proc. Ind. Acad. Sci.*, B, **14**, 201–34.

3. —— and Mathur, P. B. (1938) "Preliminary experiments on the physiology of sugarcane," *Proc. Soc. Biol. Chem. India*, **3**, 22–24.

A CONTRIBUTION TO THE EMBRYOLOGY OF *LOBELIA TRIALATA* BUCH.-HAM.

By S. B. Kausik and K. Subramanyam

Department of Botany, Central College, Bangalore

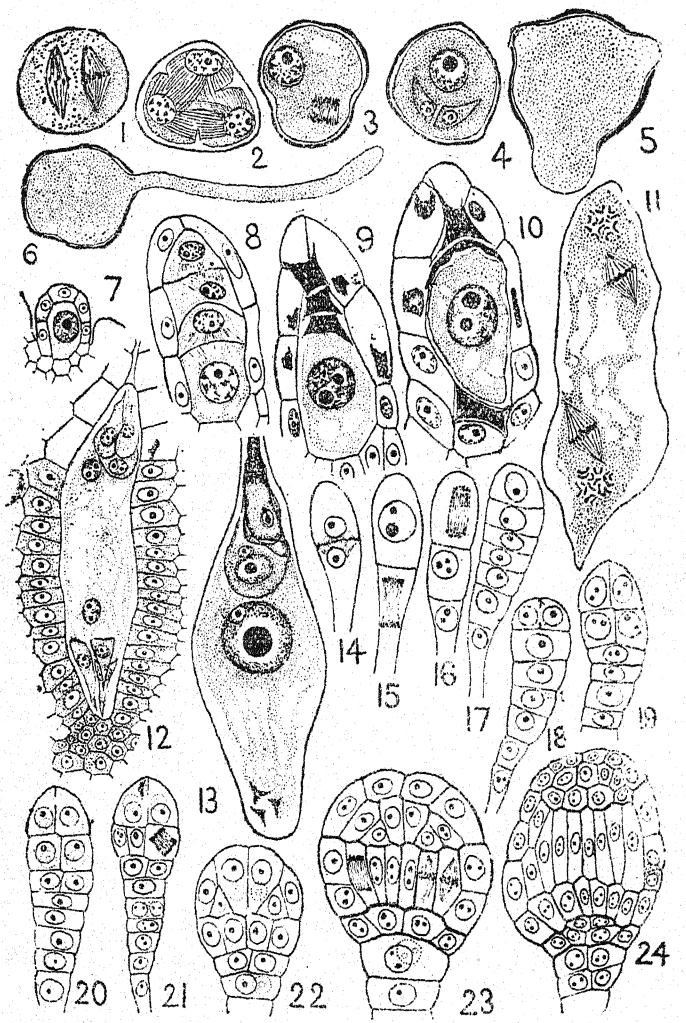
Received for publication on July 8, 1945

In a recent paper of ours on *Isotoma longiflora* Presl. (1945), we have already mentioned the literature relating to the two closely allied families Campanulaceæ and the Lobeliaceæ. In the present study we have selected for investigation a member of the latter family, *Lobelia trialata* Buch.-Ham. and the results obtained now and in our previous work on *Isotoma longiflora* of the Campanulaceæ have been so encouraging that we feel that a comparative study of these two families from an embryological point of view is likely to prove very interesting and useful. Therefore, it is proposed to investigate the life-histories of as many of the available plants as possible belonging to the families in question.

Lobelia trialata Buch.-Ham. is a glabrous annual growing to a height of fifteen inches, with winged stems and pale blue flowers. The material for study was collected at Nandi Hills, thirty-five miles from Bangalore. The subsequent processes of killing and preparation for study were employed according to the usual methods. To facilitate slight hardening of the flowers and young fruits, at the 70% alcohol stage, they were immersed in Carnoy's fluid for forty-five minutes. Sections were cut 10–24 μ in thickness and stained in Heidenhain's iron-alum hæmatoxylin with eosine as counterstain for contrast.

The Male Gametophyte

The development of the pollen grains is quite normal. In the formation of the pollen tetrads, the two division spindles in the mother cells are arranged either parallel to each other (Fig. 1), or one at right angles to the other (Fig. 2), so that both the isobilateral and tetrahedral forms are met with. The separation of the tetrads takes place by means of peripheral furrows (Fig. 2), which gradually grow towards the centre in the mother cells. The older pollen grains show the two walls clearly (Figs. 3 and 4), the outer thick exine and the inner, rather delicate intine. At this stage were seen in some preparations a large prominent nucleus, the tube nucleus, and a smaller one, the generative nucleus, which was already in the stage of telophase (Fig. 3) during the course of forming the two daughter nuclei which become organised as the two male cells (Fig. 4). The two male cells are especially clear with their cytoplasm sharply marked out from the adjacent mass of the general cytoplasm filling the whole pollen grain.



Figs. 1–24.—Fig. 1. The metaphase spindles arranged in a parallel manner for the second division in the microspore tetrad formation. ×900. Fig. 2. The formation of peripheral furrows between daughter nuclei. ×900. Fig. 3. The large tube nucleus, and the generative nucleus in the telophase stage. ×900. Fig. 4. Pollen grain at the time of shedding showing the large tube nucleus and two small male cells. ×900. Fig. 5. Pollen grain showing three germ pores. Note in one of them the pollen tube has emerged slightly. ×900. Fig. 6. Germination of a pollen grain *in situ* within the anther loculus. ×900. Fig. 7. Portion of an young ovule to show the megaspore mother cell and the integument. ×400. Fig. 8. Second division in the formation of a linear tetrad. ×900. Fig. 9. Enlarging chalazal megaspore and the degenerating upper three megaspores. ×900. Fig. 10. A linear tetrad in which the third megaspore has enlarged. ×900. Fig. 11. Formation of the four-nucleate to the eight-nucleate embryo-sac. ×900. Fig. 12. Fully organized embryo-sac showing the egg apparatus, the polars and the antipodal

cells. Note also the integumentary tapetum. ×630. Fig. 13. A stage showing double fertilization. ×630. Figs. 14–24. Development of the embryo. The primary walls indicated by thicker lines are clearly seen in Figs. 23–24. Figs. 14–21, ×450; Figs. 22 and 23, ×630; Fig. 24, ×450.

There are for each pollen grain three well-defined germ pores (Fig. 5) and in a few anther locules it was seen that the grains had already germinated *in situ* and put out a germ tube through one of the pores. It was, however, rather difficult to make out any contents in such germinating pollen grains and it is, therefore, hard to say anything definite about the fate of such pollen grains which show, peculiarly enough, this precocious tendency in germination. One such pollen grain with a fairly well-developed tube is shown in Fig. 6.

Development of the Embryo-sac

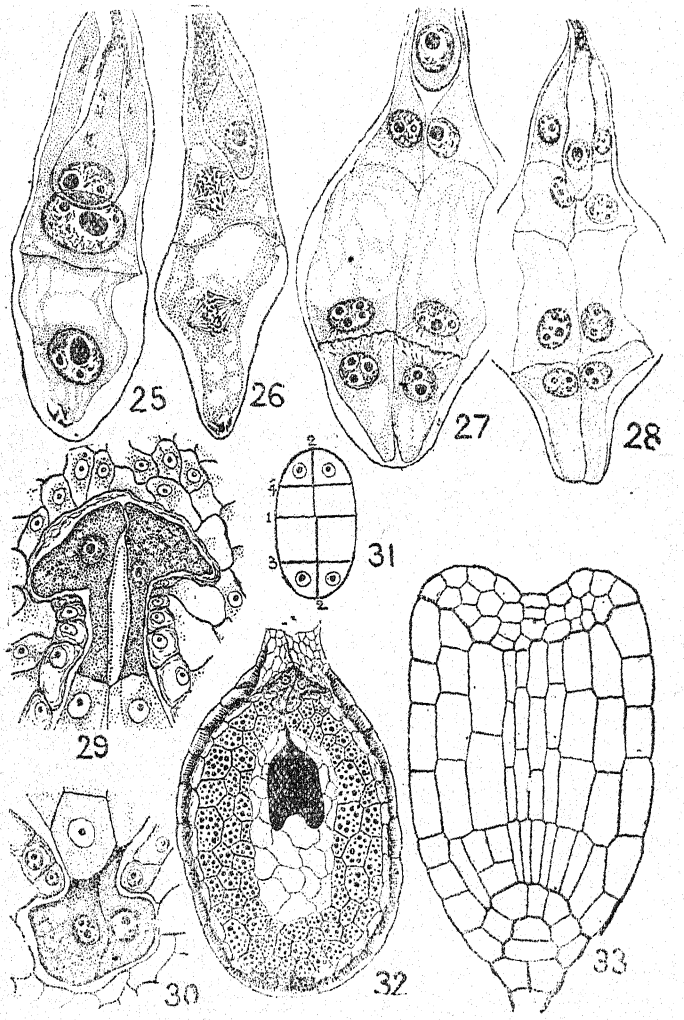
The ovary is bicarpellary and contains a large number of anatropous ovules attached on a central placenta. The ovules arise as small nucellar primordia in which gradually the single massive integument is differentiated. The nucellus is small and shows a large hypodermal archesporial cell which functions directly as the megaspore mother cell (Fig. 7). This latter cell undergoes the usual meiotic divisions and forms a linear tetrad of megaspores (Figs. 8 and 9). Usually the lowest megaspore develops further to give rise to the embryo-sac and the upper three soon degenerate, but occasionally some preparations revealed that the third megaspore may enlarge (Fig. 10). In *Lobelia nicotianæfolia* Heyne, Kausik (1938) has recorded a case where the micropylar megaspore enlarges instead of the usual chalazal one. The subsequent development of the functioning megaspore is normal (Fig. 11), and at the final stage the embryo-sac is typically eight-nucleate. The fully organized embryo-sac is long and tapering at both ends (Fig. 12), and is invested along the sides by the integumentary tapetum. The synergids are pear-shaped and show a small lateral beak just beneath the apex. The two polar nuclei which are at first seen close to the egg-apparatus and the antipodals, migrate later to the centre of the embryo-sac where they fuse immediately to form the large fusion nucleus. The antipodals are organized as definite cells with their lower ends tapering into points and degenerate at about the time of fertilization, when they are seen as darkly stained crushed remnants (Fig. 13).

One of the synergids is destroyed when the pollen tube enters the embryo-sac. Syngamy and triple fusion occur quite normally, the two processes being almost synchronous (Fig. 13).

Embryo

The fertilized egg cell becomes rapidly elongated and the first division of its nucleus takes place followed by the formation of a transverse wall (Fig. 14). Similar transverse walls are laid during subsequent divisions so that finally there results a long and slender proembryo with a number of cells (Figs. 15–17). The first cell of this proembryo then divides by a vertical wall (Fig. 18), which is next

followed by the division of the second proembryonal cell by a similar vertical wall (Fig. 19). Thus a group of four cells is formed and these in turn divide by a second set of vertical walls at right angles to the



Figs. 25–33.—Figs. 25–28. The primary divisions of the embryo-sac in the formation of the endosperm and the haustoria. Figs. 25–27, ×630. Fig. 28, ×450. Figs. 29 and 30. The two-celled micropylar and the chalazal haustoria respectively in an advanced condition. ×450. Fig. 31. Diagrammatic scheme showing endosperm development. Fig. 32. Longitudinal section of a mature seed, showing the dicotyledonous embryo, the starch-filled endosperm, the persisting micropylar and chalazal haustoria and the thick-walled outermost layer of cells. ×120. Fig. 33. A mature dicotyledonous embryo—the thicker lines are the primary walls ×450.

first set, so that now the embryonal mass consists of eight cells (Fig. 20). In the meantime, the third cell of the proembryo also divides by a vertical wall (Fig. 20). It appears that the next one or two proembryonal cells also undergo similar divisions later (Figs. 21 and 22), and thus the embryo is formed not from a single terminal cell of the proembryo, but by quite a number of these cells, so that only a few cells are finally left out to constitute the suspensor.

With the formation, as stated above, of the group of eight cells distally in the proembryo, periclinal walls arise in these cells to form the dermatogen, and still later, after further divisions, the periblem and the plerome are differentiated (Figs. 21–23). At this stage more divisions have taken place in the third embryonal tier (Fig. 24) (derived from the third cell of the proembryo) where, consequently, a group of small cells is formed. The embryo is now large and spherical, and further development proceeds rapidly. Even in later stages when the cotyledons are beginning to grow out, the initial walls of the young embryo forming the different tiers can be clearly seen, and it is, therefore, possible to assign hese tiers to the different primary body regions of the embryo, namely, the cotyledonary tier, also forming the stem tip in the notch between the two cotyledonary lobes, the hypocotyl with its central row of long and narrow plerome cells and the outer zone of much larger periblem cells, and the basal region of the embryo where the radicle is organized (Fig. 33).

ENDOSPERM

The divsiion of the primary endosperm nucleus takes place long before the zygote divides. Following the first division of the nucleus at the centre of the embryo-sac, a transverse wall is laid to form the two primary chambers in the sac (Fig. 25). There is next formed in each of these chambers a vertical wall to form two upper and two lower cells (Fig. 26), and subsequently these pairs of cells become divided transversely, first the lower pair (Fig. 27) and then the upper, so that the mbryo-sac now shows eight cells arranged in four tiers of two cells each (Fig. 28). Of these cells, the uppermost two cells constituting the first tier at the micropylar end of the sac are formed into the micropylar haustorium, and similarly the last pair of cells which makes up the fourth tier at the antipodal end develope into the chalazal haustorium. The remaining four cells belonging to the second and third tiers form the endosperm tissue. The development of the endosperm is thus according to the *Scutellaria*-type of Schnarf (1931), and the sequence of the divisions is shown diagrammatically in Fig. 31.

The micropylar haustorium is two-celled (Fig. 29), each forming laterally a prominent hump and containing a conspicuous nucleus and a finely-vacuolate dense mass of cytoplasm. The haustorium remains active for a long time in the seed (Fig. 32). The chalazal haustorium is also two-celled with prominent contents (Fig. 30). The haustorium is bulbous and seems to stop its haustorial function earlier than the micropylar haustorium. The large mass of endosperm cells filling the cavity of the seed is packed with large grains of starch, except for the

cells lying in the neighbourhood of the developing embryo (Fig. 32). The seed coat is fairly rigid and hard on account of numerous thickenings that are formed in the outermost layer of cells.

CONCLUSION

During the course of the present investigation we have been able to follow in detail certain interesting features in the life-history of this plant. These have already been dealt with in sufficient detail in the foregoing sections of the paper, but to bring together here again the more important facts about them, they are briefly the following. The germination of the pollen grain *in situ* within some of the anther locules appears to be most surprising and interesting, but unfortunately, we are not able to do anything more than merely state this fact and leave out of consideration any discussion on the probable factors that may be responsible for such a condition and also the fate of such germinating pollen grains.

There is nothing unusual in the development of the embryo-sac to merit any further comment here on the observed facts. The formation of the embryo is, however, interesting, and there are, as already described in this paper, important departures from the more normal type where only the terminal cell of a long and filamentous proembryo develops into almost the whole of the embryonal mass. Here it is found that at least the first four cells of the proembryo together contribute to the formation of the embryo, and that further, there is also seen even in very late stages a definite and clear tier-formation corresponding to these initial cells.

The development of the endosperm and the separation of the haustoria conform to the *Scutellaria*-type and it is worthwhile recalling here that a similar course is met with in certain other species belonging to the genus *Lobelia*. According to Hewit's (1939) statement on *L. amœna* the same course is probably met with as already suggested by Maheshwari (1944 *b*) in his note on *L. trigona* Roxb., and we have also very recently come to the same conclusion. It, therefore, appears quite safe to infer that the development of endosperm follows a precise and uniform course in these investigated species of *Lobelia* and is possibly also seen in the other species of this genus. If this should be so, then it becomes quite evident that the constitution of the family Lobeliaceæ as a distinct one from the family Campanulaceæ is, on embryological grounds, quite justifiable as we have already pointed out in our work on *Isotoma longiflora* (1945), which belongs to the Campanulaceæ and where the development shows certain essential departures from the *Scutellaria*-type.

In conclusion, we wish to thank Dr. L. N. Rao, Professor of Botany, Central College, Bangalore, for the many kind courtesies extended to us. We are also thankful to Mr. S. N. Chandrasekhara Iyer, Systematic Botanist, Coimbatore, for kindly determining the species.

SUMMARY

1. The pollen grains at the time of shedding are three-celled. In some anther locules the pollen grains showed germination *in situ.*

2. Megasporogenesis proceeds normally and the embryo-sac is formed according to the Normal-type. Antipodals are formed into definite cells, but these degenerate at the time of fertilization.

3. The fertilized egg develops into a long filamentous proembryo in which the uppermost three or four cells take part in the organization of the embryo. Corresponding to these cells, there is an evident tier-formation even in late stages of the embryo.

4. The primary endosperm nucleus divides much earlier than that of the fertilized egg. After the first transverse wall in the centre of the embryo-sac to form upper and lower primary chambers, subsequent vertical and transverse divisions occur in both the chambers. Thus an eight-celled tissue results, of which the micropylar pair of cells forms a two-celled micropylar haustorium, and likewise the antipodal pair forms a two-celled chalazal haustorium. The development of endosperm corresponds, therefore, to the *Scutellaria*-type of Schnarf.

LITERATURE CITED

Hewitt, W. C. (1939) .. "Seed development of *Lobelia amœna*," *Jour. Elisha Mitchel Sci. Soc.*, **55**, 63–82.

Kausik, S. B. (1938) .. "Gametogenesis and embryogeny in *Lobelia nicotianæfolia* Heyne," *Jour. Ind. Bot. Soc.*, **17**, 61–68.

—— and Subramanyam, K. (1945) "An embryological study of *Isotoma longiflora* Presl.," *Proc. Ind. Acad. Sci.*, **B**, **21**, 269–78.

Maheshwari, P. (1944) .. "The origin of the haustoria in the ovule of *Lobelia*," *Curr. Sci.*, **13**, 186–87.

Schnarf, K. (1931) .. *Vergleichende Embryologie der Angiospermen*, Berlin.

SOME FOSSIL LEAVES OF THE ULMACEÆ FROM THE KAREWA DEPOSITS OF KASHMIR

By G. S. Puri

Department of Botany and Geology, Lucknow University

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Introduction

The material described in this paper is mainly derived from collections made at Laredura partly by Dr. R. R. Stewart in 1935, and partly by the author in 1939. There are also two leaf fragments gathered by Dr. H. de Terra from Gogajipathri and Liddarmarg; and one leaf impression collected by Stewart in 1936 from Ningal Nullah. The plant-bearing outcrops lie in the Baramulla-Gulmarg region and are assigned the Lower Pleistocene age (De Terra and Paterson, 1939).

This paper has been written under the guidance of Prof. B. Sahni, F.R.S., to whom I am highly indebted for his ready help and criticism. For financial help I am grateful to the Vice-Chancellor, University of the Panjab, Principal Jodh Singh of the Khalsa College, Amritsar, and the authorities of the Lucknow University.

List of the Species

Species	Localities	References to Figures
Ulmus Wallichiana	Laredura, 6,000 ft.	Figs. 1, 2
Ulmus campestris ..	Gogajipathri, 8,800 ft. and Laredura, 6,000 ft.	Figs. 3, 4, and 8
Ulmus laevigata ..	Laredura, 6,000 ft. and Ningal Nullah, 9,000 ft.	Figs. 5, 6, 7
Ulmus sp. ..	Liddarmarg, 10,600 ft.	Figs. 9, 10

Description

Order : Urticales.

Family : Ulmaceæ.

The family is represented in the Karewa flora by a number of leaf impressions, all referable to the modern genus *Ulmus*. The genus *Ulmus* probably had a fairly wide distribution in the Valley during the Lower Pleistocene, which is evidenced by the discovery of one or the other species from four different localities including Laredura, Ningal Nullah, Gogajipathri and Liddarmarg. The genus includes three species

two of which, *viz.*, *Ulmus Wallichiana* Planch. and *U. lævigata* Royle are discovered together from the beds at Laredura. The third species, *Ulmus campestris* Linn., has been found from Gogajipathri. Fruits of *Ulmus* spp. are small and are provided with circular wings, like samaras of *Litchi*. They are likely to be represented in the fossil beds as they are capable of flight over long distances like the samaras of *Acer* and *Fraxinus*, which have also been discovered in the beds that have yielded leaves of these genera, but a very close study of the present collections has failed to bring to light any fruits of this genus.

Genus *Ulmus* Linn.

The genus includes three well determined species, the modern representatives of which are still growing in the Kashmir Valley and the nearby regions. One more leaf fragment, which could not be determined specifically, is also described.

Key to the Species

I. *Leaves large—*

(*a*) Base very oblique ; laterals 15–17 pairs, running straight and parallel .. .. *U. Wallichiana*

(*b*) Base not so oblique, leaves comparatively smaller ; laterals 12–13 pairs, curved and not strictly parallel. *U. campestris*

II. *Leaves much smaller, base rounded or cordate—*

Laterals about 10 pairs running straight and parallel..... *U. lævigata*

Ulmus Wallichiana Planch.

(Pl. XI, Figs. 1–2)

Plate XI, Figs. 1–2 are two natural size photographs of a leaf and its counterpart ; a small piece from the base on left-hand side in one specimen (Pl. XI, Fig. 1) got chipped off while splitting the clay to expose the two counterparts. The leaves are elliptic oblong in outline and measure 3·3 inches long by 1·2 inches in the broadest part, which is midway between the base and apex. The lamina, which is uniformly broad for most of its length, narrows down into a wedge-shaped base, and ends towards its upper part into a slightly curved acute apex. Margins are not well preserved but their dentate nature is clearly seen towards the apical part in Fig. 1. A small curved petiole almost complete in one leaf and slightly broken in its counterpart is also preserved.

The venation is strict-pinnate and reticulate. A stout midrib arises from the oblique base and runs in the lamina gradually thinning out towards the apical part. It follows a curved course dividing the lamina into unequal halves. 16–17 secondaries, which are about half as thick as the midrib, diverge from the latter, on either side, at acute angles. The origin of the laterals, especially in the lamina above the middle, is mostly alternate, but some laterals towards the lower portion tend to become sub-opposite ; they run straight to the

margins, parallel to one another, and each ends in a marginal tooth. The laterals, as well as the midrib, have left shallow grooves in the impression (Fig. 1) and stand out in the form of ridges in its counterpart. A comparison with modern leaves of this species shows that the former is an impression from the lower surface of the leaf whereas its counterpart represents the upper surface. The tertiary ribs are not well developed. The finer reticulation consists of a well preserved net-work of small, oval, or circular meshes, which are seen at some places in Fig. 2.

The fossils are identical with modern leaves of *Ulmus Wallichiana* Planch., a common western Himalayan elm.

Number of specimens.—Ten only.

Occurrence.—Laredura, at 6,000 ft. in the Pir Panjal Range, Kashmir.

Collection.—R. R. Stewart, 1935.

Registered numbers of figured specimens.—Pl. XI, Fig. 1=L 620 ; Pl. XI, Fig. 2=L 621.

Ulmus lævigata Royle

(Pl. XI, Figs. 5-6 ; Pl. XII, Fig. 7)

This species is based on a number of leaves, two of which are illustrated in natural size in Pl. XI, Figs. 5, 6. The figured leaves are ovate in outline measuring 1·15 inches long by ·35 inch in the broadest part, which lies midway between base and apex. One leaf (Pl. XI, Fig. 5) is slightly broken on one side along the margin. This specimen was coated in field with a thick layer of rubber solution, which has completely obscured the finer reticulations. The margins are mostly broken in the impressions but their serrate nature in one leaf (Pl. XI, Fig. 6) is clearly seen at some places in Fig. 7, which represents a part of the leaf enlarged to five diameters. Base is rounded, or slightly sub-cordate. Apex is acute.

The venation is strict-pinnate and reticulate. A fairly strong midrib runs in the lamina gradually thinning out towards the apical part. It usually runs straight but in Fig. 5 it follows a slightly curved course in a part of the lamina. 10–13 pairs of laterals, which are almost as thick as the midrib, diverge from it at acute angles ; they run straight in the lamina parallel to one another ; their manner of origin is mostly opposite, but some of them arise rarely in an alternate manner. The laterals usually end in the marginal teeth, which are mostly sharp pointed. The tertiary and finer reticulations are greatly obscured in one leaf and are badly preserved in the other, but the finer reticulation is seen at some places in the enlarged photograph.

The fossils are identical with *Ulmus lævigata* Royle, the small leaved elm of the Punjab Himalayas.

Number of specimens.—Eight only.

Occurrence.—Laredura, at 6,000 ft. and Ningal Nullah, at 9,000 ft. Pir Panjal Range, Kashmir.

Collection.—R. R. Stewart, 1935.

Registered numbers of figured specimens.—Pl. XI, Fig. 6=N 161 Pl. XI, Fig. 5=N 186.

Ulmus campestris Linn.

(Pl. XI, Figs. 3, 4 and Pl. XII, Fig. 8)

Two leaf fragments on which this species is based are illustrated in Fig. 3 on Plate XI and Fig. 8 on Plate XII ; one leaf is slightly broken at apex and along one side and the other fragment is broken at the base and both margins. The leaf lamina, which probably had an elliptic-oblong shape, narrows down to a slightly cuneate and oblique base. The narrowed nature of the lamina towards the upper part of the leaf is suggestive of its having an acute apex. The fragments vary only very slightly in size and the larger measures 2·5 inches long by 1·4 inches in the broadest part, which lies midway between base and apex. The margins seem to be biserrate.

The venation is strict-pinnate and reticulate. A fairly stout midrib runs in the lamina from the base gradually thinning out towards the apical part, and seems to divide the lamina into slightly unequal halves. 10–11 Secondaries, which are hardly half as thick as the midrib, diverge from the latter on either side at different angles. The lower pairs of laterals diverge at open angles, whereas the upper few pairs arise at acuter angles. The origin of the laterals is opposite as well as alternate ; they tend to run parallel to one another in the lamina, and end in the marginal teeth. The tertiary ribs are generally not well preserved but they are seen faintly in one specimen. Plate XI, Fig. 4, which is a photograph of a part of the leaf enlarged to about five diameters, shows a nicely preserved network of fine rectangular or pentangular meshes of the finer reticulation.

The fossils on account of their shape, size, number of laterals, etc., are identified with living leaves of *Ulmus campestris* Linn. to which they are identical in all respects.

Number of specimens.—Two only.

Occurrence.—Gogajipathri, at 8,800 ft., and Laredura, at 6,000 ft., in the Pir Panjal Range, Kashmir.

Collections.—H. de Terra, 1932 and G. S. Puri, 1940.

Registered numbers of figured specimens.—Pl. XI, Fig. 3=Loc. 2 G 6 ; Pl. XII, Fig. 8=L 823/2.

The Fourth Species

(Pl. XII, Figs. 9 and 10)

In addition to the three above described species there is one more leaf fragment, which resembles *Ulmus Wallichiana* Planch. in some features and in others it compares with *Ulmus campestris* Linn.; there are, however, a few additional features, which are entirely new to either of the two species. Pl. XII, Fig. 9 is a natural size photograph of the fragment, which is badly broken on apex, base and the two margins ; its venation is very clearly preserved and characteristic of the

genus. The laterals are seen in the photograph in the form of well marked ridges, which show that the fossil is probably an impression from the upper surface of the leaf.

The features of this leaf are compared to the modern leaves of the two fossil species in a tabular form below :—

Characters	*Ulmus* sp. (Pl. XII, Figs. 9–10)	*U. Wallichiana* Planch. (Pl. XI, Figs. 1, 2)	*U. campestris* Linn. (Pl. XI, Figs,3, 4 and Pl. XII, Fig. 8)
1. Shape of the lamina	Not known exactly but resembles more with *U. campestris* Linn. than the other species.	Elliptic oblong	Probably elliptic
2. Numer of the laterals	About 8 pairs in the fragment, which is almost complete and a complete leaf might have, at the most, three or four pairs more.	16–17 pairs.	10–11 pairs.
3. Nature of the laterals (i) angle of origin (ii) manner of divergence	Arise at acute angles and resemble *U. Wallichiana* in this feature; they are curved and do not run parallel.	Arise at acute angles; they are straight and run parallel.	Arise at comparatively less acute angles, and the lower pairs arise at open angles; they are curved and do not run exactly parallel.
(iii) Branches of laterals	Some laterals give off branches near the margin.	Laterals do not give off branches.	One or two laterals give off branches near the margins.
	Secondaries arise at unequal distances.	Arise at equal distances.	Do not arise at equal distances.
Tertiary ribs	Very well marked out and conspicuous, form cross-ties, or sometimes large, rectangular meshes; they are about half as thick as the laterals.	Not seen in a large part of the leaf; in modern leaves they are thinner than the laterals and are not at all well marked out.	Not seen in the fossil, but in modern leaves they are as conspicuous as in *U. Wallichiana*.
5. Finer reticulations	Meshes smaller, like *Ulmus Wallichiana*.	Meshes small.	Meshes comparatively larger.

From the above comparison it seems that this leaf cannot be placed in either of the two species; therefore, it is described separately as *Ulmus* sp., which is different from all modern species of *Ulmus* represented in the flora of the Himalayas.

Number of specimen.—One.

Occurrence.—Liddarmarg at 10,600 ft., in the Pir Panjal Range, Kashmir.

Collector.—H. de Terra, 1932.

Registered number of the figured specimen.—Loc. 3 L 100.

Modern Distribution of the Ulmaceæ

The family Ulmaceæ, which includes 130 species distributed in 13 genera of modern plants is, at the present time, well represented in the tropical and extra-tropical regions of the world. The most northerly point of its occurrence in the New World is 43° 30′, whereas it reaches 58° N. and 66° 59′ N. in Asia and Europe respectively. The chief genera are *Ulmus*, *Celtis* and *Trema*.

The genus *Ulmus*, with 18 species, is distributed mainly in the North temperate zone, and occurs also in the mountainous regions of tropical Asia. Two species, namely, *Ulmus montana* and *U. campestris* are typically British ; the latter is also found in north-western Europe and western Asia, and occurs in India probably as a cultivated tree at Ghoragali in the Murree Hills.

In India we have only five species—of which two (*Ulmus Wallichiana* and *U. lævigata*) are common elms of the Western Himalaya ; one species (*U. lancifolia*) is the Eastern Himalayan elm found in Sikkim, Bhutan, Assam, Chittagong and Burma ; *U. parvifolia* an evergreen shrub, occurs wild, according to Brandis, in Nubra, northern Kashmir ; the fifth Indian species is *U. campestris*, the common elm of Europe, which occurs in Baluchistan and the Kurram Valley.

Modern Distribution of the Fossil Species

The Karewa flora includes four species of *Ulmus*, three of which are definitely determined but one leaf-impression could not be specifically determined on account of its fragmentary nature.

Ulmus Wallichiana—the common West Himalayan elm—is a large deciduous tree, which occurs from the Indus to Nepal at 3,500 to 10,000 ft. (not in gregarious patches), among the coniferous as well as broad-leaved forests. It usually grows in moist ravines but it is not uncommon on dry slopes, where it is mostly stunted. It is able to colonise landslips, banks of ravines and other waste places with amazing rapidity.

In the " mixed coniferous forests of Grahan Nal," Parbatti Valley, Punjab, *Ulmus Wallichiana* occurs at 7,000 ft. to 9,000 ft. in association with *Cedrus Deodara*, *Abies Pindrow*, *Picea morinda*, *Juglans regia*, *Corylus colurna*, *Celtis australis*, *Acer* spp., etc. (Champion, 1936, p. 243).

It also occurs in the "eastern oak—fir forest of Garhwal Himalaya" with *Abies Pindrow*, *Picea morinda*, *Quercus semecarpifolia*, *Q. dilatata*, *Aesculus indica*, *Acer Cæsium*, *Corylus colurna*, *Rubus niveus*, *Rosa macrophylla*, *Skimmia Laureola*, *Syringa Emodi*, *Viburnum* spp., *Hedera Helix*, etc., etc. (Champion, *loc. cit.*, p. 245).

The "moist temperate deciduous forests of Dwali", Western Almora division, Kumaon, are composed of *Ulmus Wallichiana*, *Aesculus*

indica, *Acer Cæsium*, *A. pictum*, *Carpinus viminea*, *Betula alnoides*, *Juglans regia*, *Fraxinus micrantha*, *Quercus semecarpifolia*, *Corylus colurna*, *Cornus macrophylla*, *Rhus punjabensis*, *Taxus baccata*, *Berberis* sp., *Prunus undulata*, etc., etc. (Champion, *loc. cit.*, pp. 257–58). At Saran, in the Kagan Division of Hazara, *Ulmus Wallichiana* is associated with *Juglans regia*, *Acer Cæsium*, *Aesculus indica*, *Prunus padus*, *Pinus excelsa*, *Viburnum fœtens*. The same forests of the Sutlej Valley in the Punjab comprise of *Ulmus* spp., *Acer Cæsium*, *A. pictum*, *A. villosum*, *Aesculus indica*, *Betula alnoides*, *Carpinus* sp., *Celtis australis*, *Fraxinus micrantha*, *Juglans regia*, *Pyrus lanata*, *Prunus cornuta*, *Abies Pindrow*, *Cornus* sp., *Corylus colurna*, *Rhododendron arboreum*, *Rhus* sp., *Viburnum* spp., etc., etc. (Champion, *loc. cit.*, p. 258).

In Kashmir the species occurs in the Valley proper, Gurez, the Jhelum Valley, Keran, Kishtwar, Marwa Dacchan, Muzaffarabad, Ramban, and the Sindh Valley.

The second Karewa species—*Ulmus lævigata* (*U. villosa*)—is the small-leaved elm of the Punjab Himalaya, which occurs at lower elevations than the former species. In the valleys of Punjab rivers it ascends to as high as 10,500 ft., but usually it is not commonly found above 7,000 ft. It is fairly common in Kashmir occurring in the Valley proper, and Kamraj, Kishtwar, Marwa Dacchan, Muzaffarabad, Ramban and the Sindh Valley. Eastwards it occurs at Munali, Kulu and extends as far as the Pabar Valley. It is also found in Hazara and Murree Hills, the adjoining regions of Kashmir.

The third Karewa species—*U. campestris*—which is the common elm of Europe, occurs in India in the Kurram Valley at 7,000–9,000 ft. and in Baluchistan. It is cultivated in Kashmir and also in Ghoragali in the Murree Hills.

SUMMARY

1. The family Ulmaceæ is represented in the Karewa flora (Pleistocene) of the Kashmir Valley by four species belonging to the single genus *Ulmus*; three of these, namely, *U. Wallichiana*, *U. lævigata*, and *U. campestris* are based on leaf impressions collected by De Terra, Stewart and the author from Laredura, Ningal Nullah and Gogajipathri; the fourth is an incompletely determined species, based on a leaf fragment collected at Liddarmarg by De Terra in 1932.

2. At the present time the family, with its 13 genera and 130 species of modern plants, is distributed in the tropical and extra-tropical parts of the globe, whereas the genus *Ulmus* is mainly represented in the north temperate zone; however, some species are also found in the mountainous regions of tropical Asia.

3. Of the three species described in this paper, two (*U. Wallichiana* and *U. lævigata*) are found in the Western Himalaya, while the third (*U. campestris*), an European elm, occurs in the Kurram Valley, and is also cultivated in Kashmir and Ghoragali in the Murree Hills. The former two species are also common in Kashmir occurring in the Valley and other parts of the Jammu and the Kashmir Territories.

LITERATURE CITED

Champion, H. G. (1936) .. "A preliminary survey of the Forest types of India and Burma," *Ind. For. Rec.*, New Series, **1**, *Silviculture*, pp. 243, 245 and 248.

De Terra, H. and T. T. Paterson (1939) "Studies on the Ice Age in India and associated human *cultures*," Carnegie Institution, Washington.

EXPLANATION OF PLATES

All figures in Plates XI–XII are from untouched negatives. Figured specimens are preserved in the Botany Museum, University of Lucknow.

PLATE XI

Ulmus Wallichiana Planch.

Fig. 1. Leaf fragment (impression of the lower surface). R. R. Stewart collection, L 620 Laredura, 6,000 ft. Nat. size.

Fig. 2. Counterpart of Fig. 1, L. 621. Nat. size.

Ulmus campestris Linn.

Fig. 3. Fossil leaf impression. H. de Terra collection. Loc. 2 G. 6 Gogajipathri, 8,800 ft. Nat. size.

Fig. 4. A part of the leaf (marked ×× in Fig. 3) enlarged to show meshes of the tertiary and finer reticulations. ×*Ca.* 5.

Ulmus lævigata Royle.

Fig. 5. Fossil leaf. R. R. Stewart collection. L 186 Laredura, 6,600 ft. Nat. size.

Fig. 6. Leaf impression. R. R. Stewart collection. N 161 Ningal Nullah. Nat. size.

Fig. 7. A part of the leaf (marked ×× in Fig. 6). Enlarged to show serrate margin; meshes of tertiary and finer reticulation. ×*Ca.* 5.

PLATE XII

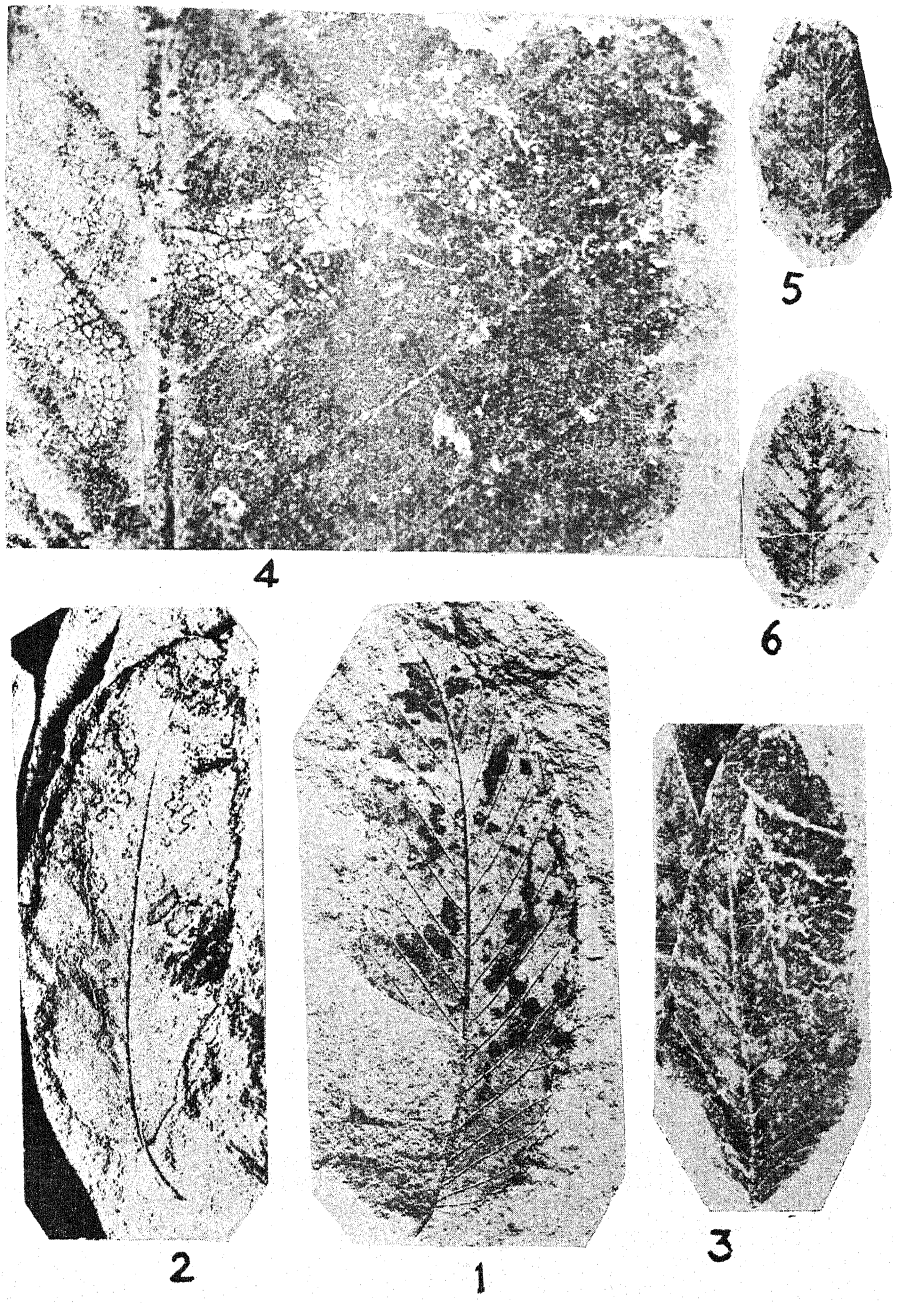
Ulmus campestris Linn.

Fig. 8. Leaf impression. G. S. Puri collection. L 823/2 Laredura, 6,000 ft. Nat. size.

Ulmus sp.

Fig. 9. Leaf impression. H. de Terra collection. Loc. 3 L 100 Liddarmarg, 10,600 ft. Nat. size.

Fig. 10. A part of the leaf (marked with ×× in Fig. 9) enlarged to show tertiaries and finer reticulations. ×*Ca.* 5.



G. S. PURI—

SOME FOSSIL LEAVES OF THE ULMACEÆ FROM THE KAREWA DEPOSITS OF KASHMIR

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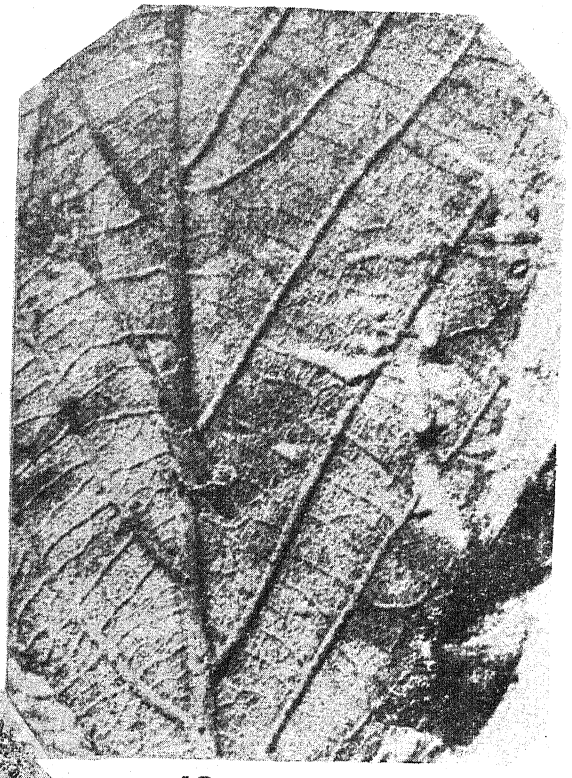

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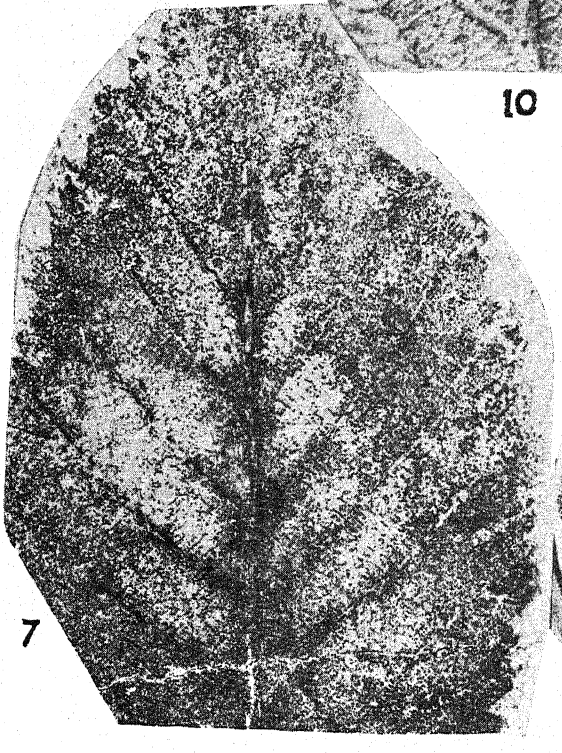

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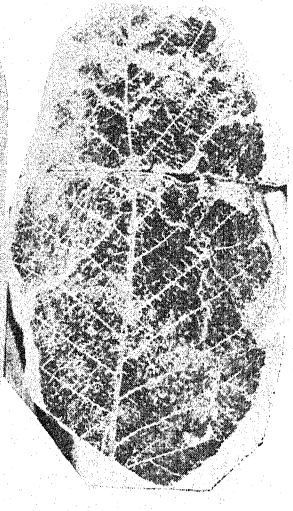

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G. S. PURI—

SOME FOSSIL LEAVES OF THE ULMACEÆ FROM THE KAREVA DEPOSITS OF KASHMIR

THE ORIGIN, DEVELOPMENT AND MORPHOLOGY OF THE OCHREA IN *POLYGONUM ORIENTALE* L.

By Gopal Chandra Mitra

Botanical Laboratory, Presidency College, Calcutta

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Introduction

Ochrea (or Ocrea) is a distinctive character of the family *Polygonaceæ*, but its morphology is not exactly clear. Asa Gray (1879) describes it as a pair of stipules united *inter se* in a sheath. Goebel (1905) considers ochrea as "axillary stipules", while Jackson (1916) defines it as "a tubular stipule, or a pair of opposite stipules so combined". Sinnott and Bailey (1914) explained ochrea as " a row of adjacent stipules each opposite one of the numerous leaf-trace bundles, which have become fused together". In view of these differences of opinion it was considered worth while to investigate the morphology of the ochrea in *Polygonum orientale* L. from the organisational and developmental points of view.

Material consisting of growing apices from adult plants was fixed in FAA either directly or after pre-treatment for half an hour in Carnoy's fluid. Sections were cut 8 μ–10 μ thick, stained with Safranin-Fast Green, Safranin-Hæmatoxylin (Heidenhain's) combinations, or with Fast Green alone.

External Morphology

The leaves of *Polygonum orientale* are arranged in two-fifth phyllotaxy. They are 10–22·5 cm. long, 5–12·5 cm. broad, petiolate, ovate-cordate, entire, acute and softly hairy on both sides. The ochrea is 1·75–3 cm. long and from its point of insertion completely envelops the next higher internode of the growing shoot. It consists of two portions, the lower sheathing base and the free tubular upper region (Fig. 1, *sh.* and *fr.*). The sheathing base is about 0·9–1·5 cm. long, is markedly striated, green, hairy on the outside and completely encircles the stem. The free tubular upper region of the ochrea is faintly nerved, grey with hairs and ends in a spreading recurved margin with a distinct notch on the side facing the leaf. The sheathing base persists as a brown scaly membrane after the leaf-fall, while the tubular upper region withers away. The terminal bud forms a small cone covered by the ochreas of several successive leaves. Here the lamina of any leaf is not protected by its own ochrea, but by that of the next

older one. The axillary buds are protected by the sheathing portion of the leaf-base until they grow beyond it.

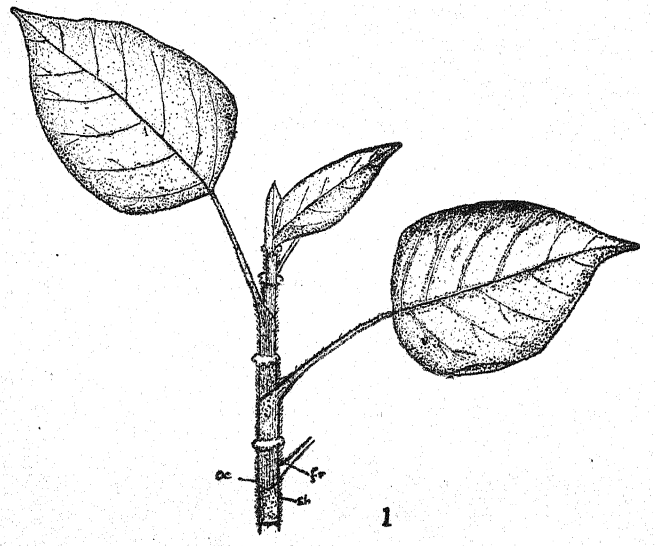


Fig. 1. *Polygonum orientale*.—A part of the shoot showing the ochrea (*oc*); *fr*, free tubular upper region; *sh*, sheathing base of a leaf. ×1.

Cellular Organisation at the Shoot Apex

The free apex of a vegetative bud of *Polygonum orientale* is relatively low and broad, and is asymmetrical with reference to the longitudinal axis (Fig. 2). A cross-section just a little below the extreme tip, shows in the first plastochrone the central axis and the base of a leaf-primordium in the form of a broad expansion on one side of the axis (Fig. 3, *a–d*).

Above the last leaf-primordium, the free apex is composed entirely of *eumeristem* (Fig. 2). The outer three or four layers form a typical *tunica*, the cells of which divide only by anticlinal walls, and within this is the *corpus*, a group of central initial cells, in which, particularly at the flanks, occasional periclinal divisions take place (Büder, 1928; Schmidt, 1924). The central initial cells are larger than the other cells of the *eumeristem*; the protoplasts appear finely vacuolated and stain lightly. The *flank* meristem surrounds the central initials as a ring of tissue, broadest at the free surface of the apex and narrowest near the pith-end of the central initial group. The cells are more regular than those of the central initials and the protoplasts are less vacuolated. In the third internode near the base, the vacuolating dividing cells tend to assume a *file* organisation and in the fourth and fifth internodes from the apex, which are elongating vary rapidly, these cells are fully organised in *files*, and their later divisions take place exclusively by transverse walls. This region corresponds to the *Rippen* meristem of Schüepp (1926).

The asymmetrical growth of the shoot apex is due to more intense activity in the sector which develops into the 'foundation' of the leaf-primordium, and comparatively less active growth in the rest of the apex.

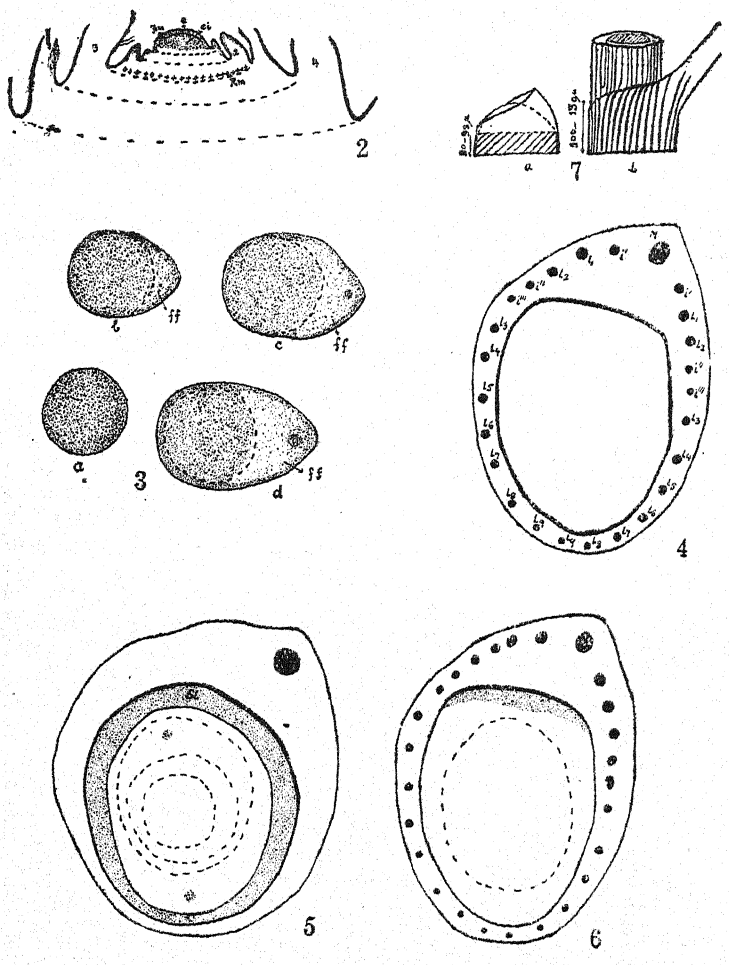
Origin of Leaf-Primordium and its Sheathing Base

The localised activity of the *flank*-meristem causes an outward radial expansion of this particular sector of the shoot apex on a buttress of vacuolating meristem (Fig. 3, *c* and *d*). This is the first definite indication of the appearance of a new primordium. This radial expansion of the growing apex has been designated by Grégoire (1935) and Louis (1935) as "*Soubassement foliaire*", by Foster (1939) as "*foliar buttress*" and by Majumdar (1942) as "*foliar foundation*".

The *foliar foundation* is seen to surround the axis quickly by the lateral spread of similar divisions along the outer two or three layers of the *flank* meristem in the form of a collar. At the end of the second plastochrone the collar has completely surrounded the axis (Fig. 3, *d*). By this time the median prodesmogen strand (which develops later into the median bundle of the leaf-trace) is seen to differentiate in the middle of the *foliar foundation*, and it runs into the free portion of the primordium as it is erected. It may then be justifiably concluded that the emergence of the leaf-primordium is influenced by the basifugally differentiating vascular system from the adjoining part of the axis (*cf.* Esau, 1942, 1943 ; Majumdar, 1945).

Development of the Main, Lateral and Intermediate Vascular Strands in the Sheathing Base

As stated above the median bundle of the leaf-trace appears as a prodesmogen strand for the first time in the second primordium. This is about 100 μ below the growing point. In the third primordium, 110μ below the growing point, near the level of insertion, the xylem is seen to differentiate in the median strand for the first time. Its further differentiation is both upwards in the free primordium and downwards in the axis. The median strand is then flanked by a lateral strand on either side (Fig. 4). Each of these lateral strands is then followed successively by two more lateral strands on each side, thus forming the second and third lateral pairs. An intermediate strand on each side then appears between the median and the first pair of laterals. Then follows the fourth pair of laterals. Between the second and the third laterals, on each side, now appear intermediates in succession. In this way fifth to ninth pair of laterals are differentiated one by one. Laterals of each opposite pair appear almost simultaneously. The sheathing bases comprising the wings and central region of the fourth and fifth primordia possess as many as 25 or 27 bundles. In older leaves further branching of the lateral and intermediate bundles is seen to take place and the total number of bundles in each sheathing base may reach 33 or more.



Figs. 2–7. *Polygonum orientale*.—Fig. 2. Longitudinal section of a vegetative bud showing shoot apex organisation. *t*, tunica; *ci*, central initial; *Fm*, flank meristem; and *Rm*, rippen meristem. ×80. Fig. 3, *a–d*. Serial transverse sections of the vegetative apex showing free apex (*a*), the initiation of the foliar foundation (*b*), and its lateral extension along the free apex (*c* and *d*). *ff*, foliar foundation. ×150. Fig. 4. T.S. of the vegetative apex showing development of the lateral and intermediate bundles in the sheath. *L*, lateral; *i*, intermediate; and *M*, median. ×130. Figs. 5 and 6. Serial transverse sections of the vegetative apex showing separation of the sheath from the axis. Fig. 5 shows the "separation tissue" (*SL*), and Fig. 6 shows remnants of this tissue adhering to the free sheath. Fig. 5, ×300; Fig. 6, ×130. Fig. 7, *a* and *b*. Diagrammatic figure showing the growth of the sheath with the axis, then its separation from the axis, its free upgrowth, and its separation from the petiole.

FURTHER GROWTH OF THE SHEATHING BASE AND ITS SEPARATION FROM THE AXIS

The sheathing base of the youngest primordium remains attached to the axis for 90–98 μ before it separates from the latter. Meanwhile the cells at the base of the third internode begin to divide actively. Soon the daughter cells organize into the *file* meristem and rapid elongation of the internode starts carrying the sheath with it.

While the internode is rapidly elongating a layer of vacuolating cells, opposite the median trace bundle of the sheath close to the axis, becomes meristematic and extends laterally around the axis to form ultimately the adaxial epidermis of the sheath. The walls of these cells become thickened and cutinised. The tissue lying between the axis proper and the newly formed adaxial epidermis of the sheath is composed of vacuolating parenchymatous cells, and is called here the 'separation layer' (*cf.* the *absciss layer* at the base of the leaf at the time of its fall), because this layer by its gradual disorganisation brings about the separation of the sheath from the axis (Fig. 5, *sl.*). It appears that the disorganization of the cells of the "separation layer" is brought about by two factors : (1) the cutting off of food supply from the sheath (which alone contains the conducting strands) due to the formation of cutinised adaxial epidermis, and (2) too much strain put on these cells on account of the very rapid extension of the leaf-primordium. The remnants of the disorganised " separation layer" remain in contact with the adaxial epidermis of the elongating sheath for some time (Fig. 6).

THE GROWTH OF THE UPPER FREE PORTION OF THE OCHREA AND ITS SEPARATION FROM THE PETIOLE

The sheathing base pursues its free upward growth for 10 to 38 μ and then separates from the petiole to continue its upward growth as the free portion of the ochrea (Fig. 7, *a* and *b*). By the time the sheathing base begins its free growth upwards all the bundles of the sheath with the exception of the median one with its first pair of laterals (these enter the petiole direct), change their upward course and follow an oblique horizontal course towards the base of the petiole from the two wings of the sheath. In their horizontal or transverse course through the sheath the bundles unite and anastomose to form one or two irregular transverse strands (Fig. 8).

The organization of the transverse strands in the wings and petiolar region of the sheath may be regarded as the end of the *first phase* and the beginning of the *second phase* in the development of the ochrea in *Polygonum orientale*. If there had been no upward growth of the sheathing base beyond the petiole, the whole structure would have remained as the sheathing base only. But in this case, where the sheathing base is to develop as an ochrea, the upward growth is not arrested with the passing of the bundles of the sheath into the petiole, as happens in *Heracleum* and other plants with leaves having sheathing bases.

A little above the transverse strand in the petiolar region of the sheath, a few cells, opposite the median bundle and between the transverse strand and the adaxial epidermis, begin to divide rapidly by longitudinal walls. This results in the differentiation of a layer of meristematic cells, three or four cells below the epidermis (Fig. 9, *ml.*). This meristematic layer then gradually extends laterally towards the edges of the sheath which it reaches ultimately.

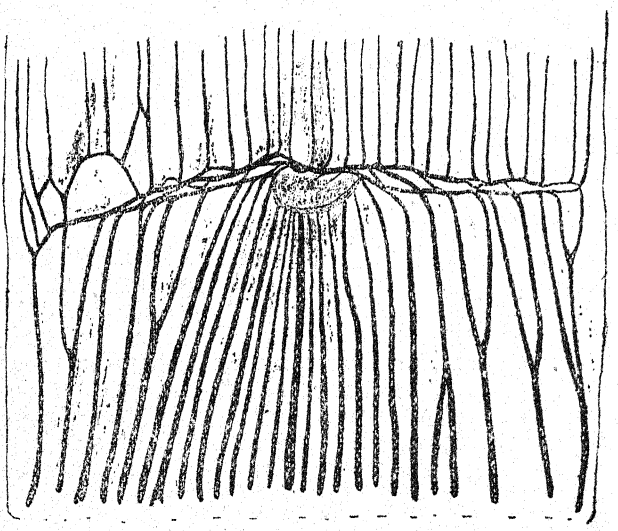


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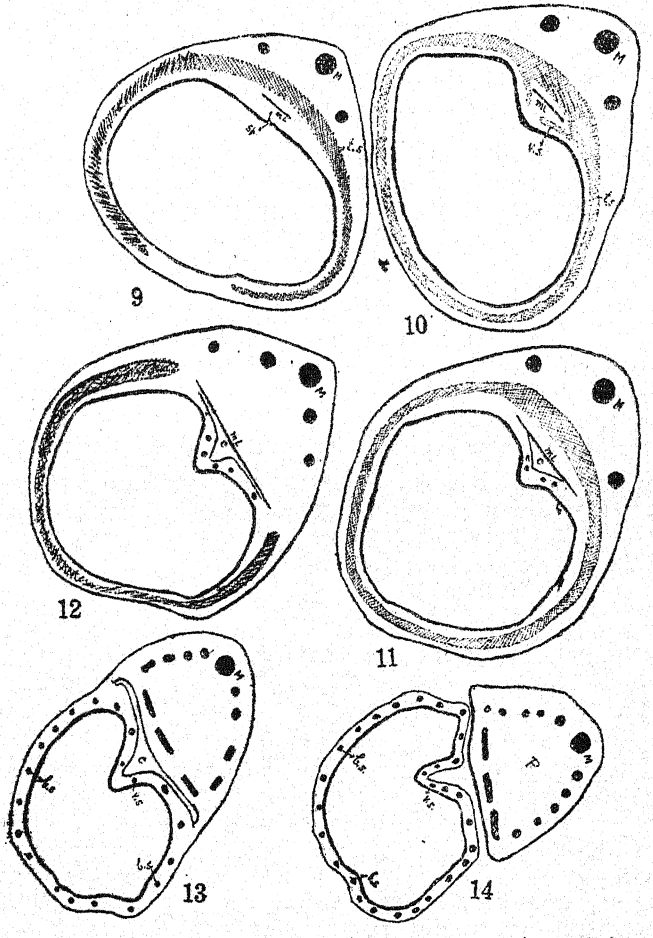
Fig. 8. *Polygonum orientale.*—An ochrea split open longitudinally, treated with chloral hydrate and stained in Safranin to show the course of vascular strands in the sheath and their branches in the upper free portion of the ochrea. ×9.

While the meristematic layer is extending laterally, its cells towards the epidermis begin to expand radially pushing the cells in front and stimulating them to activity. As a result the sheath in front of this layer splits into two parts and for some time the split-ends move apart not being able to keep pace with the lateral extension of petiolar base (Fig. 9).

Meanwhile the meristematic cells within this redially expanding layer become gradually transformed into the adaxial epidermis of the petiolar base. This newly formed epidermis cuts off food supply to the radially extending cells immediately in its front, with the result that these cells disorganise. Thus a small cavity originates opposite to the median bundle; this separates the adaxial epidermis of the petiole from the abaxial surface of the sheathing base now beginning to separate from the petiole (Figs. 10 and 11). The outermost cells of this region of the sheath then gradually change into the characteristic abaxial epidermis of the sheath.

The meristematic layer extends laterally till the petiole is entirely separated from the sheath (Figs. 12 and 14). The sheath now separated from the petiole grows upwards as the free upper portion of the

ochrea, and the split noticed in the early stages of separation and which is seen to grow from 10–30 μ in length is later closed. This split appears as the notch in the mouth on the posterior side of the fully developed ochrea (Fig. 9). It will thus be seen that the fork or notch does not indicate the composition of the ochrea as Asa Gray (1879, p. 106)



Figs. 9–14. *Polygonum orientale.*—Serial transverse sections of the vegetative bud showing separation of the ochrea from the petiole. Fig. 9. Origin of the posterior notch in the mouth of the ochrea; shows appearance of a meristematic layer opposite the median bundle and the split caused by radial expansion of the tissue in front of the meristematic layer. ×120. Fig. 10. Shows the vascular supply from the petiolar base to the portion of the ochrea in front of the petiole. ×80. Fig. 11. Shows the formation of the cavity and the lateral extension of the meristematic layer. ×80. Figs. 12–14. Show gradual extension of the meristematic layer and the cavity resulting in the complete separation of the ochrea from the petiole. ×80. *ml*, meristematic layer; *Sp*, split; *M*, median; *ts*, transverse strand; *VS*, vascular strand from the petiolar base to the ochrea in front; *c*, cavity; *bs*, branch strand from the transverse strand to the ochrea; and *P*, petiole.

would describe it, but is the result of pressure put on the sheath cells by the radially expanding cells of the petiole.

The stimulus which the cells of the separating portion of the sheath get from the radially expanding cells just in front of the meristematic layer, causes rapid growth and extension of this region and a fold soon appears opposite the petiolar base, evidently for accommodation of the upper part of the ochrea in the vegetative bud (Figs. 11–14).

Vascular Supply of the Ochrea above the Base of the Petiole

While the bundles in the sheath are changing their course towards the base of the petiole and organising themselves into obliquely horizontal strands in the upper portion of the sheath, branches are given off from these strands (and in some cases also from the sheath bundles before these change their course) on the adaxial side of the sheath. Two or three bundles of the free upper portion of the ochrea which appear later in front of the petiole are secondarily derived from the transverse strand in the petiolar end of the sheath before it separates from the petiole (Figs. 8 and 10). The free upper portion of the ochrea gets about 22 to 25 bundles from the sheath, but in the mature condition the number is seen to increase to 29 or more due to the branching of these bundles in the free portion.

The free upper portion is comparatively thinner than the sheathing base. This appears to be due to the fact that the branches which are given out on the adaxial side are much slender than the sheath bundles from which they are given out.

Discussion

The leaf of *Polygonum orientale* has a sheathing base, which originates independently of the nodal topography. Innervation of the base takes place in the second and third plastochrones. The upward growth of the sheath, till it separates from the axis, is due partly to the stimulus of growth and food material supplied through the large number of prodesmogen strands in different stages of differentiation. Further upward growth of the sheath is influenced, as suggested by Sinnott and Bailey (1914), by the stimulus of growth and food supply carried forward by the trace bundles till it is separated from the petiole.

In the case of leaves having sheathing bases alone, as in *Heracleum* and other plants, the lateral trace bundles in the sheath change their vertical course and run obliquely into the base of the petiole. The stimulus for upward growth of the sheathing base and food supply for influencing it being no longer available it remains as the sheathing base only. In the case of the ochrea of *Polygonum orientale* on the other hand, slender branches are given off from the lateral trace bundles during their united horizontal course towards and through the base of the petiole. These branches conduct the necessary food and stimulus for the continuance of the upward growth of the sheath, but in an attenuated form.

The view, most widely accepted and generally cited in text-books, about the morphology of the ochrea, which is a distinct feature of the leaf of *Polygonaceæ*, is that it represents two united stipules. This view is largely based on comparative morphology. The present investigation shows that the ochrea consists distinctly of two portions. The lower portion agrees in its development with the sheathing leaf-base such as is seen in *Heracleum* (*cf.* Majumdar, 1942). It is distinguished externally in *Polygonum orientale* as described above by its green colour and prominent striations. The upper tubular portion is of the nature of an outgrowth from the leaf-base. As the stipules are generally regarded as outgrowths of the base of the leaf primordium (Sinnott and Bailey, 1914), the upper portion of the ochrea can also be interpreted to be of a stipular nature. The investigation, however, provides no evidence to show that it consists of two opposite stipules. It also contradicts the conclusion of Sinnott and Bailey (1914) that it is formed from a row of adjacent stipules each opposite one of the leaf trace bundles which have become fused together.

Summary

The vegetative bud of *Polygonum orientale* has been studied from the organisational and developmental points of view. It shows that the ochrea has got two parts : (1) the sheathing base (which is found in many other groups of flowering plants) and (2) the free upper portion which develops as an outgrowth of the sheathing base and therefore, is to be interpreted as of stipular nature. The study does not support the conclusion of Sinnott and Bailey (1914) that the ochrea is a product of the fusion of a row of adjacent stipules.

Acknowledgments

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LITERATURE

Büder, J. (1928) .. "Der Bau des phanerogamen Sprotzvegetationspunktes und seine Bedeutung für die Chimärentheorie," *Ber. dtsch. b. Ges.*, **46**, 20–21.

Esau, K. (1942) .. "Vascular differentiation in the vegetative shoot of *Linum*: I. The Procambium," *Amer. Jour. Bot.*, **29**, 738–47.

——— (1943) .. "Origin and development of primary vascular tissue in seed plants," *Bot. Rev.*, **9**, 125–206.

Foster, A. S. (1938) .. "Structure and growth of the shoot apex in *Ginkgo biloba*," *Bull. Torr. Bot. Club*, **65**, 531–56.

——— (1939) .. "Structure and growth of the shoot apex of *Cycas revoluta*," *Amer. Jour. Bot.*, **26**, 372–85.

Gray, A. (1879) .. *Structural Botany*, Vol. I, London.

Goebel, K. (1905) .. *Organography of Plants*, Part II, 1st Edition, English Translation, London.

Gregoire, V. (1935) .. "Donnees nouvelles sur la morphogenese de l'axe feuillé dans les Dicotylées," *C. R. Acad. Sci., Paris*, **200**, 1127–29.

Jackson, B. D. (1916) .. *A Glossary of Botanical Terms*, London.

Louis, J. (1935) .. "L'Ontogénèse du système conducteur dans la pousse feuillé des Dicotylées et des Gymnosperms," *La Cellule*, **44**, 87–172.

Majumdar, G. P. (1942) .. "The Organisation of the Shoot in *Heracleum* in the Light of Development," *Ann. Bot.*, N.S. **6**, 49–81.

——— (1945) .. *Presidential Address, Section of Botany, Proc. Ind. Sci. Congress.*

Priestley, J. H. (1928) .. "The Meristematic Tissue of the Plant," *Biol. Rev.*, **3**, 1–20.

Schmidt, A. (1924) .. "Histologische Studien an phanerogamen Vegetationspunkten," *Bot. Arch.*, **8**, 345–404.

Schuepp, O. (1926) .. "Meristeme Linsbauer," *Handb. d. Pflanzenanatomie*, Abt. **1**, Teil 2.

Sinnott, E. W. (1914) .. "The anatomy o the node as an aid in the classification of Angiosperms," *Amer. Jour. Bot.*, **1**, 303–22.

—— and Bailey, I. W. (1914) "Investigations on the Phylogeny of the Angiosperms, Nodal Anatomy and the Morphology of Stipules," *ibid.*, **1**, 441–53.

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UNIVERSITY OF LUCKNOW,
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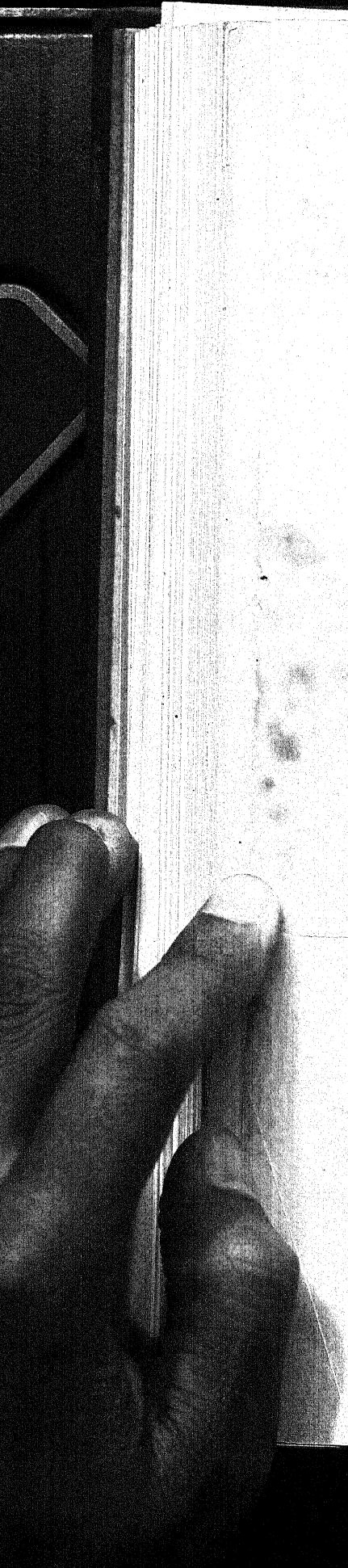
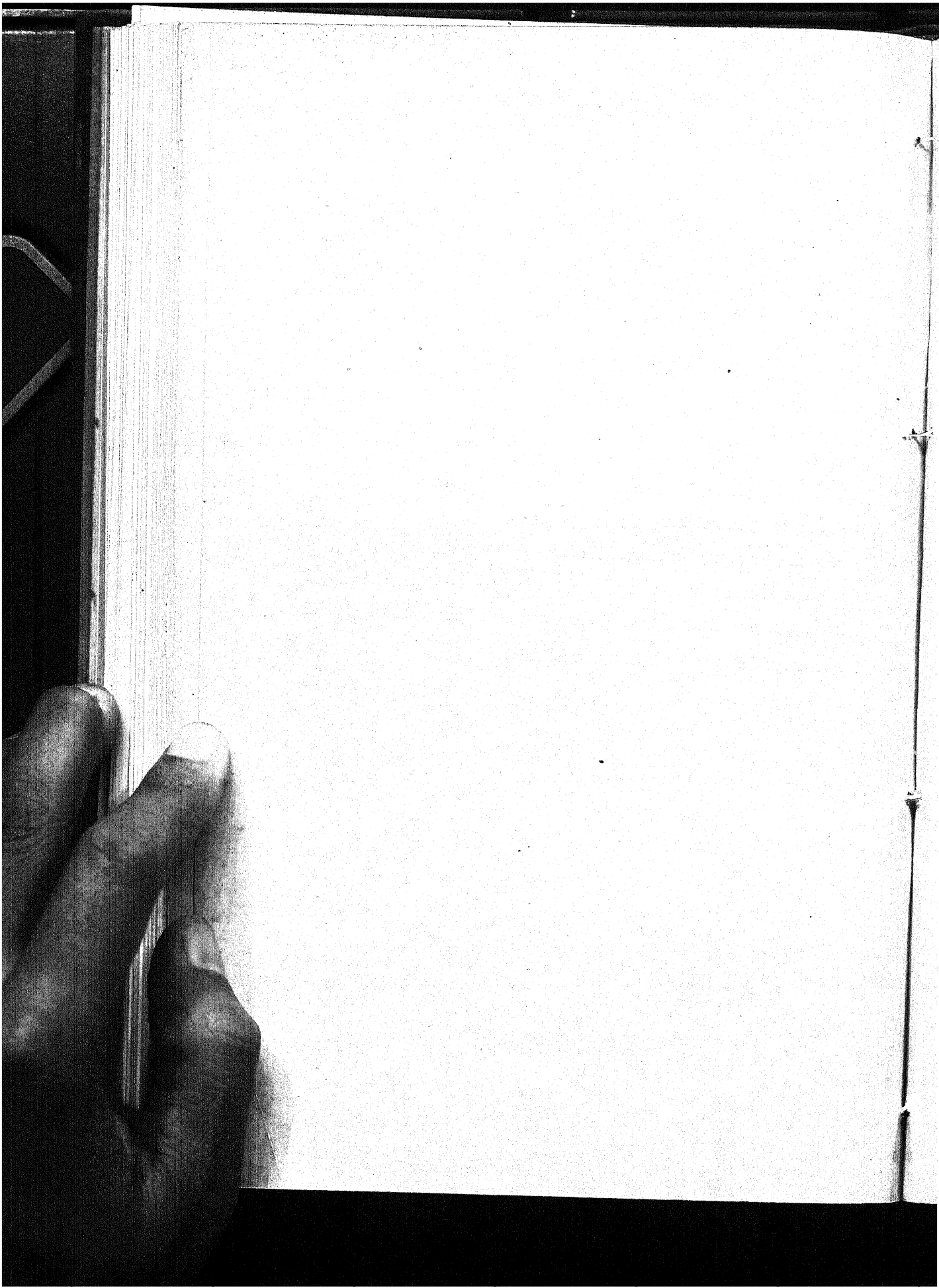


CONTENTS



RULES OF THE INDIAN BOTANICAL SOCIETY

(With amendments up to 3-1-1944)

Founded December 6, 1920

(Registered under Act XXI of 1860)

The Indian Botanical Society had its inception in a resolution passed by the Botany Section of the Indian Science Congress at the Nagpur meeting in January 1920. A Committee of Organisation was consequently formed to carry this resolution into effect. This committee consisted of the late Dr. P. Bruhl of the University College of Science, Calcutta, the late Rao Bahadur K. Rangachariar of the Agricultural College, Coimbatore, the late Rai Bahadur Prof. Shiv Ram Kashyap of the Government College, Lahore, Prof. Birbal Sahni, then of the Benares Hindu University, Benares, Dr. W. Burns, then of the College of Agriculture, Poona, and the late Dr. Winfield Dudgeon of the Ewing Christian College, Allahabad, with Dr. Dudgeon as Chairman.

In October 1920 the Committee sent out a letter to as many botanists as could be located in India, inviting them to become charter Members of the new Society. It was agreed that 25 members would be considered sufficient for founding the Society and that office-bearers should be elected when this number was reached. The response to this invitation was so immediate and hearty that it was possible to hold elections for office-bearers of the Society by about the middle of November. Upon completion of the election on December 6th, the Society was declared duly organised, and the Committee of Organisation ceased to exist.

NAME, PURPOSE AND ACTIVITIES

1. The Society shall be called the Indian Botanical Society.

2. The purpose of the Society shall be to promote the cause of Botany in India in all its aspects.

3. The Society shall attempt to achieve this purpose

   (*a*) By publishing a Botanical Journal.

   (*b*) By holding general and local meetings with a view to diffusing botanical knowledge among the public and facilitating intercourse between members.

   (*c*) By encouraging original investigations.

   (*d*) By organising efforts to create facilities for botanical work in the country.

   (*e*) By starting branches in various centres, if ten or more members apply from each centre.

   (*f*) By appointing sub-committees wherever and whenever they may be required.

MEMBERSHIP

4. Membership shall be open to all persons interested in Botany.

5. There shall be two classes of members, Ordinary and Honorary.

6. Members shall be admitted to the Society after being nominated by any two Ordinary Members and elected either by a unanimous vote of the Executive Council of the Society, or by a simple majority of the members present at an annual meeting.

7. Ordinary Members shall be entitled to all the privileges of the Society, and shall receive gratis all the publications of the Society.

8. Ordinary Members may become Life Members upon payment of Rs. 150 either in a lump sum, or in instalments within a year from the time of their application for life membership, provided that any ordinary Member who has already paid a number of annual subscriptions may be allowed a rebate at the rate of half the annual subscriptions paid, up to a maximum of *eight* years.

9. The number of Honorary Members shall not, at any time, exceed ten. Such membership shall be restricted to persons eminent for their contributions to Botanical Science. Honorary Members shall be elected after the unanimous recommendation of the Executive Council, and by four-fifths majority of those present and voting at the Annual Meeting. They shall enjoy all the privileges of Ordinary Members, without payment of fees, excepting that of holding office.

PRIVILEGES

10. All Ordinary and Honorary Members shall receive a copy of the Journal free.

11. The local branches shall receive a copy of the Journal free. They shall also have the right to admit local student-members on payment of Membership Fee of Re. 1 per annum. This money shall be utilised by the Local Branches for meeting their expenses.

12. Ordinary and Honorary Members shall have the right of communicating papers for the meetings and the Journal, and all members, including student-members, shall have the right of attending the Annual Meetings, but the student-members shall have no votes.

WITHDRAWAL OF MEMBERS

13. A Member may withdraw from the Society by signifying his wish to do so in a letter addressed to the Secretary. The Society, however, shall not be liable to return any fee that may have been paid by the member in advance.

14. A withdrawing member whose subscription is not in arrear shall automatically recover the privileges of membership without re-election if he rejoins the Society within six months of withdrawal. He shall then be liable to pay all dues as if he had not withdrawn at all.

SUBSCRIPTIONS

15. The annual subscription of Ordinary Members shall be Rs. 12-8.

16. The financial year shall begin on the 1st of October and end on 30th September, and the annual subscriptions shall be paid in advance to the Treasurer, provided that new members elected after the first of August shall be exempted from payment of dues for the closing financial year.

17. Members whose subscriptions have expired and who have not paid for the current year will receive the first number of the current volume of the Journal by V.P.P.

SUBSCRIPTIONS IN ARREAR

18. Members whose subscriptions are in arrear shall be excluded from the privileges of membership until they shall have paid the arrears A list of all such members, showing the amounts due from them shall be submitted by the Secretary to the President at each annual meeting, and it shall be read out at the request of any member present at the meeting.

19. The Treasurer shall, at the end of the financial year, send a letter or bill with a request to renew his or her subscription to every member at his or her last known address. If the subscriptions are not paid within two months of the despatch of the letter or bill, the Council may remove the member's name from the Society's register.

20. Any member whose name has been removed under the preceding rule may be re-elected on payment of his arrears.

THE MANAGEMENT

21. There shall be:—

(*a*) An Executive Council, which shall carry on all the affairs of the Society except those concerning the publication and distribution of the Journal and Proceedings.

(*b*) The Editorial Board, which shall be responsible for the publication and distribution of the Journal and Proceedings.

THE EXECUTIVE COUNCIL

22. The Executive Council shall consist of:—

(*a*) the President, (*b*) Two Vice-Presidents (one of whom shall be the retiring President of the Society), (*c*) the Secretary, (*d*) the Treasurer, (*e*) the Editor-in-Chief and (*f*) ten elected Councillors.

23. The President, Vice-Presidents and Councillors shall serve for one year each, the Secretary and the Treasurer for three years. Each year, the Councillors who have been in office for more than

three years shall retire from the Council, and shall not be eligible for re-election until after the lapse of one year from the date of their retirement. The time of retirement of office-bearers shall be immediately after the close of the Annual Meeting.

24. The President (or, in his absence, one of the Vice-Presidents) shall preside, if present, at all general meetings of the Society and shall deliver an address at the Annual Meeting at which he presides.

25. The Secretary shall perform the duties usually devolving upon that office.

26. The Treasurer shall be responsible for the financial affairs of the Society during the term of his office and shall submit by the end of October each year the accounts of the Society for the current financial year to a certified Auditor appointed by the President.

27. The time, place, and agenda of the Annual General Meeting shall be arranged by the Executive Council, as far as possible in co-operation with other organisations having a similar purpose.

THE EDITORIAL BOARD

28. The Editorial Board shall consist of :—

(*a*) Four Members elected for four-year periods and retiring in rotation.

(*b*) The Treasurer (*ex-officio*), who shall also be the Business Manager of the Journal.

(*c*) The Secretary of the Society (*ex-officio*).

(*d*) One member nominated annually by each University which contributes Rs. 150 or more to the Society annually.

29. The Editor-in-Chief shall be elected by the Editorial Board among its own members.

30. The subscription to the Journal from non-members shall be Rs. 15 or its equivalent in foreign currency.

ELECTIONS TO OFFICES

31. All elections to the office shall take place at the annual Meeting of the Society, *interim* vacancies being filled up by the Executive Council.

32. The following procedure shall be adopted for all elections to office :—The Secretary shall cause a list of members of the Society to be circulated before the 31st August in each year and invite from members entitled to vote nominations for each office falling vacant to be received before the 30th September.

The Executive Council shall also nominate one member for each office falling vacant. For this the Secretary shall cause a list of the members of the Society to be circulated before the 31st August each year and invite Executive Councillors to propose names for

each office falling vacant to be received before the 15th September. The names so proposed by the Executive Councillors shall be circulated to them for voting, and those obtaining the largest number of votes, shall be considered as nominees of the Executive Council. The voting papers from the Executive Councillors shall be received before the 7th October. A list of names proposed by the members together with the nominations of the Council shall be circulated to members before the 30th November each year.

33. All voting shall be by secret ballot on forms supplied by the Secretary and returned, as advised by him, in special envelopes provided for the purpose.

34. Votes shall be scrutinized at the Annual Meeting.

35. A majority shall elect. Tie ballots shall be decided by lot.

36. All retiring office-bearers shall be eligible for re-election, except those mentioned in Rules 20 & 23.

Amendment to the Rules

37. The above rules may be amended at any Annual General Meeting by a three-fourths majority of the members present and voting. Amendments may be proposed by any member and must reach the Secretary in time for circulation to all members of the Society at least one month before the meeting.

LIST OF THE PAST OFFICERS OF THE SOCIETY

PRESIDENTS:

1921	Professor WINFIELD DUDGEON.
1922	Rao Bahadur K. RANGACHARIAR.
1923	Professor B. SAHNI.
1924	Professor S. R. KASHYAP.
1925	Professor R. S. INAMDAR.
1926	Professor S. L. AJREKAR.
1927	Rev. Dr. E. BLATTER.
1928-29	Professor M. O. P. IYENGAR.
1930	Professor P. PARIJA.
1931-32	Professor T. EKAMBARAM.
1933	Professor S. P. AGHARKAR.
1934	Professor R. H. DASTUR.
1935	Professor J. H. MITTER.
1936-37	Professor S. R. BOSE.
1938	Professor H. G. CHAMPION.
1939	Rai Bahadur Professor K. C. MEHTA.
1940	Professor H. CHAUDHURI.
1941	Professor S. L. GHOSE.
1942	Professor M. A. SAMPATHKUMARAN.
1943	Dr. K. BAGCHEE
1944	Professor Y. BHARADWAJA (resigned in Nov.)

VICE-PRESIDENTS:

1921	Dr. W. BURNS.
1922	Professor S. P. AGHARKAR.
1923	Professor M. O. P. IYENGAR.
1924	Professor M. A. SAMPATHKUMARAN.
1925	Professor S. L. AJREKAR.
1926	Professor P. PARIJA.
1927	Dr. H. CHAUDHURI.
1928-29	Professor S. L. AJREKAR.
1930	Dr. S. K. MUKERJI.
	Dr. T. EKAMBARAM.
1931-32	Dr. S. L. GHOSE.
	Professor P. PARIJA.
1933	Professor T. EKAMBARAM.
	Professor J. H. MITTER.
1934	Professor M. O. P. IYENGAR.
	Professor J. H. MITTER.
1935	Professor P. PARIJA.
	Professor S. R. BOSE.
1936	Professor P. PARIJA.
	Dr. K. BAGCHEE.
1937	Professor B. SAHNI.
	Professor H. G. CHAMPION.

1938	Professor S. L. GHOSE.
	Professor R. H. DASTUR.
1939	Mr. H. G. CHAMPION.
	Dr. H. CHAUDHURI.
1940	Rai Bahadur Professor K. C. MEHTA.
	Dr. K. BAGCHEE.
1941	Dr. H. CHAUDHURI.
	Dr. SHRI RANJAN.
1942	Professor Y. BHARADWAJA.
	Dr. N. L. BOR.
1943	Principal P. PARIJA.
	Professor M. A. SAMPATHKUMARAN.
1944	Dr. A. C. JOSHI.
	Dr. P. MAHESHWARI

SECRETARIES AND TREASURERS:

1921-22	Professor S. R. KASHYAP.
1923	Professor R. S. INAMDAR.
	Professor B. SAHNI.
1923-28	Mr. N. K. TIWARY.

SECRETARIES:

1929-30	Mr. N. K. TIWARY.
1931	Professor WINFIELD DUDGEON.
1932-34	Dr. S. K. MUKERJI.
1934-38	Dr. E. K. JANAKI AMMAL.
1938-41	Professor Y. BHARADWAJA.
1942-44	Professor G. P. MAJUMDAR.

TREASURERS:

1929-30	Professor M. O. P. IYENGAR.
1930-33	Professor T. EKAMBARAM.
1933-43	Professor M. O. P. IYENGAR.
1944	Professor G. P. MAJUMDAR.

CHIEF EDITORS:

1921-26	Professor P. F. FYSON.
1927-28	Professor B. SAHNI.
1929-30	Professor M. O. P. IYENGAR.
1930-34	Rai Bahadur Professor S. R. KASHYAP.
1934-42	Professor P. PARIJA.
1943-44	Professor M. O. P. IYENGAR.

BUSINESS MANAGERS:

1925-30	Professor M. O. P. IYENGAR.
1930-33	Professor T. EKAMBARAM.
1933-43	Professor M. O. P. IYENGAR.
1944	Dr. A. C. JOSHI.

OFFICERS OF THE SOCIETY, 1945

President.

Dr. N. L. Bor, M.A., D.Sc., F.L.S., I.F.S, F.N.I., C.I.E.

Vice-Presidents.

Prof. H. Chaudhuri, Ph.D., D.Sc., D.I.C., F.N.I.
Dr. B. P. Pal, M.Sc., Ph.D., F.L.S.

Editor-in-Chief.

Prof. M. O. P. Iyengar, M.A., Ph.D., F.L.S., F.N.I

Treasurer.

Dr. A. C. Joshi, D.Sc., F.N.I.

Secretary.

Dr. S.N. Das Gupta, M.Sc., Ph.D., D.I.C.

Elected Members of the Executive Council.

Prof. P. L. Anand, M.Sc., Ph.D.
Prof. F. R. Bharucha, B.A., B.Sc., MSc., Dr.es Sc., F.N.I.
Prof. M. O. P. Iyengar, M.A., Ph.D., F.L.S., F.N.I.
Dr. T. S. Mahabale, B.A., M.Sc., Ph.D.
Prof. G. P. Majumdar, M.Sc., Ph.D., F.N.I., F.A.Sc.
Prof. R. L. Nirula, B.Sc., Ph.D., D.I.C.
Dr. P. Parija, M.A., D.Sc , F.N.I., I.E.S., O.B.E.
Prof. T. S. Raghavan. M.A , Ph.D., F.L.S.
Prof. L. Narayana Rao, M.Sc., Ph.D.
Prof. M. Sayeed-ud-Din, B.Sc., M.A., F.R.M.S., F.A.Sc , F.L.S.

Editorial Board.

Prof. S. P. Agharkar, M.A., Ph.D., F.N.I.
Prof. H. Chaudhuri, M.Sc., Ph.D., D.Sc., D.I.C., F.N.I.
Dr. S. N. Das Gupta, M.Sc., Ph.D., D.I.C. (Ex. Officio).
Prof. M. O. P. Iyengar, M.A., Ph.D., F.L.S., F.N.I. (Chief Editor).
Dr. A. C. Joshi, D.Sc., F.N.I. (Ex. Officio).
Dr. P. Maheshwari, D.Sc., F.N.I.
Prof. G. P. Majumdar, M Sc., Ph.D., F.N.I., F.A.Sc.

MINUTES OF THE TWENTY-FOURTH ANNUAL MEETING

(Held at Nagpur, on January 3rd, 1945)

The Twenty-fourth Annual General Meeting of the Indian Botanical Society was held on January 3rd, 1945, at Nagpur, with Dr. P. Maheshwari, Vice-President of the Society, in the Chair.

The following business was transacted :—

1. The Minutes of the last Annual General Meeting held in Delhi were read and confirmed.

2. The following resolution was moved from the Chair, and unanimously passed, all present standing :

   "That the Society records its deep sense of loss at the sad demise of Prof. M. A. Sampathkumaran (Bangalore) who was the President (1942), Vice-President (1924 and 1943) of the Indian Botanical Society and that a copy of the resolution be forwarded to the members of the bereaved family."

3. The Secretary-Treasurer read the Annual Report and the statement of audited accounts for the year ended on 30th September, 1944, and the same were passed unanimously.

4. The Secretary reported the unanimous election of the following persons as Life and Ordinary members of the Society since the last Annual Meeting :

   Life Members : Dr. R. D. Asana (Pusa, Bihar), Dr. K. Biswas (Calcutta), Mrs. E. Gonzalves (Bombay), Mr. M. B. Raizada (Dehra Dun), and Dr. H. Sitarama Rao (Dehra Dun).

   Ordinary Members : Dr. R. D. Adatia (Bombay), Dr. K. S. Bhargava (Pilani, Jaipur State), Mr. P. D. Bhate (Poona), Dr. D. B. Chatterjee (Gauhati), Dr. U. N. Chatterjee (Allahabad), Mr. K. Das (Benares), Prof. S. R. Deshpande (Poona), Mr. A. P. Mehrotra (Allahabad), Mr. A. K. Mitra (Allahabad), Mr. J. D. Oza (Ahmedabad), Dr. N. L. Pal (Allahabad), Mr. K. M. K. Reddy (Vizagapatam), Mr. G. D. Srivastava (Allahabad), Mr. V. Subramanian (Allahabad), Dr. D. D. Tiwari (Allahabad), Mr. S. D. N. Tiwari (Jubbulpore) and Mr. J. C. Varma (Allahabad).

5. The Chairman appointed Profs. F. R. Bharucha and J. C. Sen Gupta as scrutineers of ballot papers for the election of office-bearers for the year 1945. On their report the following result was announced :

6. The following budget for the year 1944-45, as presented by the Treasurer, was adopted :

Receipts :

	Rs.	A.	P.
Opening Balance	3,209	0	8
Subscriptions :			
Ordinary members	1,575	0	0
Life members (Balance due)	142	8	0
Contribution from Universities	250	0	0
Rockefeller Foundation	500	0	0
Subscription to :			
Journal :			
Foreign	400	0	0
Inland	850	0	0
Bank Commission	10	0	0
Sale of Reprints	50	0	0
Sale of Back Volumes	100	0	0
Deficit over Income	1,301	14	4
Totel	8,388	7	0

Payments :

	Rs.	A.	P.
Secretary, Treasurer and Chief Editor	720	0	0
Rent	144	0	0
Postage	225	0	0
Audit Fees for 1943-44	40	0	0
Audit Fees for 1944-45	40	0	0
Bank Commission	15	0	0
Printing and Stationery general for all officers.	200	0	0
Year Book	250	0	0
Journal Vol. XXIV	3,500	0	0
Two remaining Nos. of Vol. XXIII	900	0	0
Liabilities :			
Out of Rockefeller Foundation Grant, Bangalore Press	496	3	0
*To Refund :			
Advance from Reserve Fund (Last Audit Report)	1,234	8	0
To credit to the Reserve Fund :			
Life Membership fees 1943-44	481	4	0
Life Membership fees 1944-45	142	8	0
Total	8,388	7	0

7. The question of the celebration of the Silver Jubilee of the Society in 1946 was referred to the Executive Council for the year 1945.

8. The question of the publication of the Journal was considered along with the reports of the Editor-in-Chief and the Business Manager. The Editorial Board was requested to take necessary action in order to ensure the regular appearance of the Journal once every two months.

9. As recommended by the Treasurer a sum of Rs. 48 regarded as unrecoverable was written off :

		Rs.	A.	P.	
On account of	Prof. B. N. Singh ...	29	8	0	(reprint).
,,	Mr. S. Ahmed ...	5	0	0	,,
,,	Mr. E. Nelles ...	13	8	0	
	Total ...	48	0	0	

10. The Chairman delivered his Presidential Address on "The Place of Angiosperm embryology in Research and Teaching."

11. With a vote of thanks to the retiring office-bearers, the meeting was dissolved.

P. MAHESHWARI,
Chairman,
24th Annual Meeting,
Indian Botanical Society.
3rd January, 1945.

G. P. MAJUMDAR,
Secretary,
Indian Botanical Society.

ANNUAL REPORT FOR THE YEAR 1943-44

The Executive Council of the Indian Botanical Society have pleasure in submitting the following Annual Report of the Society for the year ended 30th September, 1944:—

Membership:

At the end of the financial year the number of members of the Society was as follows:—

Ordinary members	...	115 * (13 after August)
Life Members	...	28
Honorary Members	...	8

Meetings:

The twenty-third Annual Meeting of the Society was held at Delhi on the 3rd January, 1944, in the room of the Botany Section of the Indian Science Congress with Dr. K. Bagchee, the President, in the chair.

The officers for the year 1944 were elected at the meeting. The Executive Council and the Editorial Board, including the representative from the Madras University, were constituted as follows:

THE EXECUTIVE COUNCIL

President: Prof. Y. Bharadwaja (Benares) resigned on 17th November 1944

Vice-Presidents: Dr. P. Maheshwari (Dacca)
Dr. A. C. Joshi (Benares)

Secretary: Prof. G.P. Majumdar (Calcutta)

Treasurer: Prof. M O P. Iyengar (Madras)
&
Prof. G. P. Majumdar (Calcutta)

Councillors:
Prof. S. P. Agharkar (Calcutta)
Prof. F. R. Bharucha (Bombay)
Dr. S. N. Das Gupta (Lucknow)
Dr T. S. Mahabale (Ahmedabad)
Prof. R. L. Nirula (Nagpur)
Mr P. Parija (Cuttack)
Prof. T. S. Raghavan (Annamalai)
Mr M. S. Randhawa (Rae Bareli)
Dr. T. S Sabnis (Cawnpore)
Prof. B. Sahni (Lucknow)
Dr. R. K. Saksena (Allahabad)
Prof. M. Sayeed-ud-Din (Hyderabad)

Editorial Board: Prof. S. P. Agharkar (Calcutta)
Prof. H. Chaudhuri (Lahore)
Prof. G. P. Majumdar, ex-officio (Calcutta)
Dr. P. Parija (Cuttack), resigned.
Dr. A. C. Joshi, Business Manager (Benares)
Prof. M. O. P. Iyengar, Chief Editor (Madras)
Prof. B. Sahni (Lucknow), resigned.

The Executive Council held one meeting during the year under report on the 3rd January, 1944, in the room of the Botany Section of the Indian Science Congress, Delhi.

Publications: During the year under report were published Nos. 5 & 6 and Index of Vol. XXII and Nos. 1 & 2 of Vol. XXIII of the Journal.

Subscribers (Other than members) for the Journal:

The Journal was subscribed for by 57 inland institutions and 26 foreign institutions.

Exchanges: The number of exchanges was *ten* of which *four* were foreign and *six* inland.

Library: The publications received were duly added to the Library.

Donations: Two Universities have kindly made the following annual contribution:—

Madras University ... Rs. 150
Travancore University... Rs. 100 (received on 18-10-44)
Rockefeller Foundation
New York, U. S. A.
(for 1944-45) Through
the National Institute
of Sciences of India ... Rs. 500 (received on 25-10-44)

Finance: The accounts of the Society for the year ending 30th September, 1944, were audited by Messrs. U. N. Chaudhuri & Co. of Calcutta. The Receipts and Payments accounts, as on the 30th September, 1944, submitted by the Auditors are given in Appendix A. The total actual receipts during the year were Rs. 455-7-0 (including the amount received by sale of Postal Cash Certificates of Rs. 1,234-8-0) and payments Rs. 3,542-10-3.

At the beginning of the year, the cash position of the Society was as follows:—

	Rs.	a.	p.
Post Office Cash Certificates at cost ...	6,930	0	0
Cash with the Imperial Bank of India. ...	1,936	1	6
Cheques uncashed with the Imperial Bank on 30-9-1943 ...	112	8	6
Cash with the Secretary ...	106	5	11
Cash with the Treasurer ...	46	4	0
Total ...	9,131	3	11

At the end of the year the cash position was as follows on 1-10-44:

		Rs.	a.	p.
Post Office Cash Certificates at cost	...	5,730	0	0
Cash with the Imperial Bank of India	...	2,955	4	3
Cash with the Secretary	...	1	15	0
Cash with the Chief Editor	...	101	4	2
Cash with the Business Manager	...	149	15	3
Total	...	8,939	0	8

APPENDIX A

NEW ADDITIONS OF EXCHANGE PUBLICATIONS

(Since the publication of the lists in 1938, 1939, 1940, 1941, 1942, 1943.)

34. Current Science.
Vol. XIII. Nos. 5, 6, 7, 8, 9, 10.

48. Instituto Botanico de la Universidad, Central Quito.
Boletin del-Ano 1. Num. 3 and 4 (1943)

51. The Journal of the Southern Appalachian Botanical Club (Castanea.)
Vol. IX. Nos. 1-3, 4.

54. The Journal of the Bombay Natural History Society.
Vol. XLIV. Nos. 2, 3, 4.

86. Transactions of the Royal Society of South Africa.
Vol. XXX, Part 2 (1944).

123 Lloydia (A quarterly Journal of Biological Science).
Vol. VI. No. 4, Vol. VII., Nos. 1, 2.

133 Science Museum Library (Weekly list of accessions to the Library).
Nos. 679-683; 684-687; 688-691; 692-696; 697-700; 701-704.

136 Journal of the Department of Science, Calcutta University, New Series.
Vol. I. No. 4. (1944).

126 Transactions of the Bose Research Institute, Calcutta.
Vol. XV (1942-43).

Proceedings of National Institute of Sciences, India.

Statement of Receipts and Payments Account for one

RECEIPTS	Rs. A. P.	Rs. A. P.
To Opening Balance ...	...	... 2,201 3 11
,, Subscriptions from Ordinary Members ...	997 8 0	
,, ,, ,, Life Members.	481 4 0	
		1,478 12 0
,, Advance Subscriptions from Ordinary Members ...	...	... 127 8 0
,, Subscriptions from foreign institutions ...	...	... 553 1 4
,, Subscriptions from inland institutions ...	...	... 687 0 0
,, Grants : Madras University ...	...	... 150 0 0
,, Sale proceeds of Back Vols. of Journals ...	...	... 81 14 0
,, Cost of re-prints ...	...	... 176 1 3
,, Sale of P. O. Cash Certificates	...	... 1,234 8 0
,, Difference in Exchange (foreign)	...	... 55 0 5
,, Bank Commission ...	...	... 6 10 0
Total Receipts ...	6,751 10 11	
Total Expenses ...	3,542 10 3	
BY BALANCE C. O. ...	3,209 0 8	
With I. B. P. Pk. St. ...	2,955 14 3	
,, Secretary ...	1 15 0	
,, Editor ...	101 4 2	
,, B. Manager ...	149 15 3	
Total ...	3,209 0 8	... 6,751 10 11

Auditors' Report.

We beg to report that we have examined the above statement for the year ended 30th September, 1944, with the books, counterfoil, therewith. We have obtained a certificate from Imperial Bank of value of Rs. 5,730 at cost.

G. P. MAJUMDAR,
Hony. Secretary & Treasurer.
29-11-44.

DIX B.

CAL SOCIETY

year from 1st October, 1943 to 30th September, 1944.

		PAYMENTS					
		Rs.	A.	P.	Rs.	A.	P
Treasurer:							
By House Rent	...	84	0	0			
,, Remuneration to Staff	...	175	0	0			
,, Postage	...	89	13	6			
,, Contingency	...	25	7	6			
,, Printing & Stationery	...	2,492	13	3			
,, Bank Commission	...	11	6	0			
,, Remuneration to Auditor for 1942-43	...	40	0	0			
					2,918	8	3
Chief Editor:							
By House Rent	...	60	0	0			
,, Remuneration to staff	...	125	0	0			
,, Postage	...	28	0	3			
,, Contingency	...	16	14	9			
					229	15	0
Business Manager:							
By Postage	...	24	1	0			
,, Contingency	...	9	11				
,, Establishment	...	30	0	0			
					63	12	9
Secretary:							
By Establishment	...	180	0	0			
,, Postage	...	62	6	6			
,, Printing & Stationery	...	83	15	9			
,, Contingency	...	4	0	0			
					330	6	3
				Total ...	3,542	10	3
By Cash at Bank and in hand as shown in the margin				...	3,209	0	8
				Total ...	6,751	10	11

of Receipts and Payments Account of the Indian Botanical Society receipts, and vouchers and have found the same in accordance India, Madras, the custodian of P. O. Cash Certificates of the

Dated, Calcutta,
the 29th November, 1944.
12/1, Old Post Office Street,
Calcutta.

U. M. CHAUDHURI & CO.,
Registered Accountants,
Auditors.

LIST OF MEMBERS

HONORARY MEMBERS

Date of election

1936 Blackman, Frederick Frost, D.Sc. (Lond.), F.R.S., *late Reader in Botany, University of Cambridge*, Upper Cross, Storey's Way, Cambridge, England.

1933 Bower, Frederick Orpen, M.A., Sc.D. (Cantab.), D.Sc. (Dub., Sydney, Leeds), LL.D. (Aberd., Glas., Bristol), F.R.S., F.R.S.E., F.L.S., *Emeritus Regius Professor of Botany, University of Glasgow*, 2, The Crescent, Ripon, Yorkshire, England.

1939 Buller, Arthur Henry Reginald, Dr.Phil. (Leipzig), D.Sc. (Birm.), Hony. D.Sc. (Pennsylvania), Hony. LL.D. (Manitoba and Saskatchewan), Hony. D.L. (Cal.), F.R.S., *Emeritus Professor of Botany, University of Manitoba*, Winnipeg, Canada.

1937 Darlington, C. D., Ph.D., D.Sc., F.R.S., *Director John Innes Horticultural Institution*, Mostyn Road, Merton Park, London, S.W. 19, England.

1939 Fritsch, Felix Eugen, Ph.D. (München), D.Sc. (Lond.), F.R.S., F.L.S., *The Botany School*, Cambridge, and 34 Causeway Side, Cambridge.

1932 Fyson, P. F., M.A., I.E.S. (*Retired*), C/O The High Commissioner for India, London, England.

1935 Wieland, G. R., Ph.D., *Professor of Botany, Yale University*, New Haven, Connecticut, U.S.A.

ORDINARY MEMBERS

(The names of Life Members are marked with an asterisk.)

1942 Abdul Majeed, M., M.Sc., Professor of Biology, S. E. College, Bahawalpur State, Bahawalpur, Punjab.
(*Diatoms.*)

1939 Abeywickrama, B.A., University of Ceylon, Colombo, 'Pepiliyana', Boralesgamuwa, Ceylon.
(*Ecology, Physiology, Anatomy and Economic Botany.*)

1945 Adatia, Dr. R. D., D.Sc., Professor of Botany, Wilson College, Bombay.

1945 Agarwala, Shirish Chandra, M.Sc., Research Assistant, Mango Necrosis Scheme, (I.C.A.R.), Botany Department the University of Lucknow, Lucknow.
(*Plant Pathology and Physiology.*)

Date of election

1920 *Agharkar, Shankar Purushottam, M.A. (Bom.), Ph.D. (Berl.), F.L.S., F.N.I., Ghose Professor of Botany and Head of the Department of Botany, Calcutta University, 35, Ballygunge Circular Road, Calcutta.

(*Systematic Botany, Ecology, Plant Geography, Aquatic and Marine Flora.*)

1941 Ahmad, Ghias-ud-Din, B.Sc. Agri. (Punj.), B.Sc. (Lond.), M.S. (Calif.), Bar-at-Law, Assistant Professor of Botany, Punjab Agricultural College, Lyallpur.

(*Plant Physiology, Genetics.*)

1920 *Ajrekar, Shripad Lakshman, B.A. (Bom. et Cantab), Diploma in Agriculture (Cantab), F.N.I., I.E.S. (*Retired*), Bhandarkar Institute Road, Poona 4.

(*Mycology and Plant Pathology.*)

1928 *d'Almeida, J. F. R., B.A., M.Sc., Professor of Botany, St. Xavier's College, Hill Road, Bandra, Bombay.

(*Ferns, Marsh and Aquatic Plants.*)

1939 Anand, Pyare Lal, M.Sc. (Punj.), Ph.D. (Lond.), Professor of Biology, Sanatana Dharma College, Lahore, and Lecturer in Botany, Punjab University.

(*Ecology and Taxonomy of Marine Algæ*).

1944 *Asana, Dr. Rustom D., M.Sc. (Bom.), Ph.D. (Lond.), D.I.C. (Lond.), Sugarcane Physiologist, Central Sugarcane Research Station, Pusa, Bihar.

(*Plant Physiology.*)

1928 *Bagchee, Krishnadas, M.Sc. (Cal.), D.Sc. (Lond.), D.I.C., F.N.I., Mycologist, Forest Research Institute, New Forest, Dehra Dun.

(*Cytology, Plant Genetics, Mycology and Plant Pathology.*)

1945 Bakshi Bimal Kumar, M.Sc., Lecturer in Botany, Rajshahi College, Rajshahi, Bengal.

(*Mycology and Systematic Botany.*)

1941 Banerjee, Sachindra Nath, M.Sc. (Cal.), Assistant Lecturer in Botany, Calcutta University, 35, Ballygunge Circular Road, Ballygunge, Calcutta.

(*Mycology and Plant Pathology.*)

1930 Banerji, Ilabonto, M.Sc. (Cal.), Lecturer in Botany, Calcutta University, 131, Harish Mukerji Road, Kalighat, Calcutta.

(*Cytology and Plant Breeding.*)

1926 Baria, Mrs. D. D. H. (nee Kanga), M.Sc. (Bom.), "Panorama", 203, Walkeshwar Road, Malabar Hill, Bombay 6.

(*Physiology, Histology, Morphology and Cytology.*)

Date of election

1943 Bhaduri, Paramnath, M.Sc. (Cal.), Ph.D. (Lond.), F.L.S., F.R.M.S., Lecturer in Botany, University College of Science, 35, Ballygunge Circular Road, P.O. Ballygunge, Calcutta.

(*Cyto-Genetics.*)

1920 Bhakay, Gadadhar Narayan, M.Sc. (All.), Professor of Biology, Lucknow Christian College, Lucknow.

1920 Bharadwaja, Yajnavalkya, M.Sc. (Panj.), Ph.D. (Lond.), F.L.S., F.N.I., Professor of Botany and Head of the Department of Botany, Benares Hindu University, Benares.

(*Algology, Limnology, Ecology, Bryology and Economic Botany.*)

1933 Bhargava, Hari Raman, M.Sc. (Agra), Research Student, Department of Botany, Agra College, Agra.

(*Morphology and Cytology of Angiosperms.*)

1944 Bhargava, Kameshwar Sahai, M.Sc., D.Phil (Alld.), Asstt. Professor of Botany, Birla College, Pilani (Jaipur State).

(*Cytology of Fungi, Mycology and Plant Pathology.*)

1937 Bharucha, Fardunji Rustomji, B.A., B.Sc. (Bom.), M.Sc. (Cantab.), Dr. es Sc. (Montpellier), F.N.I., Professor of Botany, Royal Institute of Science, Bombay.

(*Phytosociology, Plant Physiology, Evolution, Genetics and Systematic Botany.*)

1945 Bhate, Prabhakar D., M.Sc., Lecturer in Biology, Wadia College, Poona.

(*Bryophyta, Pteridophyta.*)

1945 Bhatt, Rama Shanker, M.Sc., Ph.D., Research Assistant, Mango Necrosis Scheme (I.C.A.R.) Botany Department, University of Lucknow.

(*Plant Pathology and Mycology.*)

1920 *Biswas, Kalipada, M.A. (Cal.), D.Sc. (Edin.), F.R.S.E., Superintendent, Royal Botanic Garden, Sibpur, Calcutta.

(*Algology, Systematic Botany and Ecology.*)

1939 Bor, Norman Loftus, M.A. (Dublin), D.Sc. (Edin.), F.L.S., I.F.S., F.N.I., C/o Conservator of Forests, Shillong, Assam.

(*Gramineae and Plant Ecology.*)

1920 Bose, Sahay Ram, M.A., Ph.D., F.R.S.E., F.N.I., Professor of Botany, Carmichæl Medical College, 1, Belgachia Road, Calcutta.

(*Anatomy and Cytology of Higher Fungi, especially Polyporaceae.*)

1943 Chakravarti, Amal Kumar, M.Sc. (Cal.), Assistant Cytologist, Banana Research Scheme, Department of Botany, Calcutta University, 35, Ballygunge Circular Road, P.O. Ballygunge, Calcutta.

(*Embryology and Cytology.*)

Date of election

1944 Chakravarty, Madhusudan, M.Sc., Research Fellow in Botany, Calcutta University, College of Science, 35, Ballygunge Circular Road, P.O. Ballygunge, Calcutta.

(*Floral Biology, Mycology and Plant Pathology.*)

1920 *Champion, Harry George, M.A. (Oxon.), C.I.E., F.N.I., Professor of Forestry, Imperial Forestry Institute, Oxford, England.

(*Ecology, Forestry and Physiology.*)

1944 Chatterjee, Dr. Debabrata, M.Sc. (Cal.), Ph.D. (Edin.), F.L.S. (Lond.), Lecturer in Botany, Cotton College, Gauhati, Assam.

(*Taxonomy, Plant Distribution, Ecology.*)

1940 Chatterji, N. K., D.Phil. (All.), Lecturer in Botany, Dacca University, Ramna P.O., Dacca.

(*Plant Physiology and Plant Pathology.*)

1945 Chatterji, Dr. U. N., B.Sc. (Hons.), M.Sc., D.Phil. (Alld.), Research Scholar, Botany Department, University of Allahabad, Allahabad.

(*Plant Physiology.*)

1943 Chaturvedi, Suraj Bhan, M.Sc., Assistant Professor of Botany, Maharaja's College, Jaipur City, Jaipur State, Rajputana.

(*Fungi, Angiosperms, Floral Anatomy and Embryology*).

1926 Chaudhuri, Haraprasad, M.Sc. (Cal.), Ph.D., D.Sc. (Lond.), D.I.C., F.N.I., Professor and Head of the Department of University Teaching in Botany and Director, Kashyap Research Laboratory, Punjab University, Lahore.

(*Plant Pathology and Plant Physiology.*)

1943 *Chavan, Appa Saheb Ram Chandra Rao, B.Sc. (Bombay), Ph.D. (Neb., U.S.A.), Professor of Biology, Baroda College, Baroda: Juna Modikhana, Baroda.

(*Ecology, Grasses, Bryology.*)

1939 Chopra, Ram Saran, M.Sc. (Punj.), Demonstrator in Botany, Punjab University, Lahore.

(*Bryology.*)

1937 *Chowdhury, Kafiluddin Ahmad, B.A. (Cal.), M.Sc. (Syracuse), D.Sc. (Edin.), F.N.I., Wood Technologist, Forest Research Institute, New Forest, Dehra Dun.

(*Anatomy, Physiology and Palæobotany.*)

1945 Chowdhury, N. P., M.Sc., Technical Assistant, Dictionary of Economic Products and Industrial Resources of India, 20, Pusa Road, New Delhi.

(*Economic Botany and Morphology.*)

1943 Chowdhury, Sudhir, M.Sc., B.Sc. (Agri.), Assoc. I.A.R.I., Plant Pathological Laboratory, Sylhet, Assam.

(*Mycology and Plant Pathology.*)

Date of election

1944 Craig, William Morison, E. C. 9462 Lieutenant, Cipher Officer, C/o Lloyds Bank, New Delhi, India.

1943 Damle, Vasudeo Purushottam, M.Sc., Lecturer in Botany, Fergusson College, Poona 4, Bombay.
(*Mycology, Physiology and Ecology.*)

1920 Das, Atulananda, F.L.S., I.F.S. (Retd.), F.R.S.E., Chief Forest Officer, Mayurbhanj State, Baripada.
(*Systematic Botany and Forest Ecology.*)

1945 Das, Kumudabhiram, M.Sc., Lecturer, College of Agricultural Research, Benares Hindu University, Benares.
(*Agricultural Botany.*)

1935 *Das Gupta, S. N., M.Sc. (Cal.), Ph.D. (Lond.), D.I.C., Reader in Botany, The University, Lucknow.
(*Mycology and Plant Pathology.*)

1922 Dastur, Rustom Hormasji, M.Sc. (Bom.), F.L.S., F.N.I., Institute of Plant Industry, Indore (Central India).
(*Plant Physiology.*)

1944 Desai, Ramakant Madhav Rao, B.Sc., M.Sc. (Bom.), Professor of Biology, Dharmendrasinghji College, Rajkot, Kathiawar.
(*Physiology, Anatomy, Ecology and Cryptogams.*)

1945 Deshpande, Shankar Rangnath, M.Sc. (Bom.), Asstt. Professor of Biology, S. P. College, Poona 2.
(*Bryophytes, Pteridophytes and Morphology.*)

1935 Dutt, Nand Lall, M.Sc. (Punj.), Government Sugarcane Expert, Imperial Sugarcane Station, Lawley Road P.O., Coimbatore.
(*Economic Botany and Genetics.*)

1941 Fotidar, A. N., M.Sc. (Benares), A.I.F.C., K.F.S., Assistant Conservator of Forests, Kamraj Division, Baramulla, Kashmir.
(*Plant Ecology, Forestry and Flora of Kashmir.*)

1943 Ganguly, Ajit Kumar, M.Sc., Lecturer-Demonstrator in Biology, Ripon College, Calcutta, 1, Sagar Dhar Lane, P.O. Beadon Street, Calcutta.
(*Anatomy, Mycology and Plant Pathology.*)

1921 Ghose, Surendra Lal, M.Sc. (Punj.), Ph.D. (Cantab), F.L.S., F.N.I., Professor of Botany, Government College and Director, University Botanical Laboratory, Punjab University, Lahore.
(*Algæ, Gymnosperms and Cytology.*)

1944 Ghosh, S. S., M.Sc., Assistant Wood Technologist, Forest Research Institute, New Forest, Dehra Dun.
(*Anatomy of Living and Fossil Plants, especially wood.*)

Date of election

1939 Ginai, M. Asgar, M.Sc. (Punj.), Assistant Mycologist, Fruit Experimental Station, Quetta.

(*Mycology, Agronomy and Horticulture.*)

1943 *Gonzalves, Mrs. Ella, B A., M.Sc., Assistant Lecturer, Royal Institute of Science, Dept. of Botany, Mayo Road, Bombay 1.

(*Algology.*)

1939 Gorrie, R. Maclagan, D.Sc., F.R.S.E., I.F.S., Forest Office, Lahore.

(*Forest Ecology, Grassland Ecology, Role of plant cover in soil erosion control.*)

1933 Gupta, Babu Lal, M Sc., (All.), Lecturer in Botany, Agra College, Agra.

(*Morphology of Angiosperms.*)

1935 Gupta, Din Dayal, B Sc. Hons. Agri. (Wales), Dy. Asst. Director of Purchase, Directorate General of Food, Jamnagar House, New Delhi.

(*Plant Breeding, Genetics, Cytology, Plant Pathology and Improvement of Grasslands.*)

1945 Hsü, Jen, Assistant Professor of Botany, National Peking University, China Department of Botany, Lucknow University, Lucknow.

(*Palæobotany and Structural Botany.*)

1939 Iyengar, C. V Krishna, M.Sc. (Mad.), Assistant Professor of Botany, Intermediate College, Mysore.

1920 Iyengar, Mandayam Osuri Parthasarathy, M.A. (Mad.), Ph.D. (Lond.), F.L.S., F.N.I., ex-Director, University Botany Research Laboratory, Triplicane P.O., Madras.

(*Algæ.*)

1943 Jacob, K. T., M.A. (Mad.), Ph.D. (Lond.), Cytogeneticist, Bose Institute, 93, Upper Circular Road, Calcutta.

(*Cytology and Genetics.*)

1922 *Janaki Ammal, Miss E. K., M.A. (Mad.), D.Sc. (Michigan), John Innes Horticultural Institution, Mostyn Road, Merton Park, London, S.W. 19.

(*Cytology, Genetics and Ecology*).

1945 *Johansen Donald A., Box 32, Stanford University, California.

1945 Johar, Dayal Singh, M.Sc. (Hons. Sch.), Microbiologist, Indian Institute of Fruit Technology, Lyallpur.

(*Plant Pathology and Microbiology of canned foods.*)

1933 Johri, Brij Mohan, D.Sc. (Agra), Dayalbagh, Agra.

(*Morphology of Angiosperms, Pteridophytes and Gymnosperms.*)

Date of election

1930 Joshi, Amar Chand, D.Sc. (Punj.), F.N.I., Professor of Botany, Benares Hindu University, Benares. (On leave); Assistant Editor, Dictionary of Economic Products and Industrial Resources of India. Council of Scientific and Industrial Research, 20 Pusa Road, New Delhi.

(*Morphology, Cytology and Economic Botany.*)

1939 Kajale, Laxman Balwant, D.Sc. (Benares), Department of Biology, T. N. J. College, Bhagalpur.

(*Morphology of Angiosperms.*)

1944 Kanitkar, Upendra Keshav, B A., M.Sc., Professor of Botany, Sri Parashuram Bhan College, Poona 2, Bombay.

(*Plant Physiology, Plant Pathology.*)

1921 Kanjilal, Praphulla Chandra, B.Sc., I.F.S., Deputy Conservator of Forests, 4, Rai Behari Lal Road, Lucknow.

(*Taxonomy, Ecology.*)

1943 Kar, Baikuntha Kumar, M.Sc. (Allahabad), Dr. Phil. (Leipzig), Plant Physiologist, Bose Institute, 93, Upper Circular Road, Calcutta.

(*Plant Physiology.*)

1925 Kausalya, Miss C. K., B.A., B.Sc (Lond.), Professor of Natural Science, Queen Mary's College, Madras.

(*Plant Pathology.*)

1943 Kausik, S. B., M.Sc., D.Sc., Lecturer in Botany, Central College, Bangalore.

(*Floral Morphology and Embryology of Angiosperms.*)

1944 Khan, Muhammadkhan Sardarkhan, B.Sc., 2544 Jogawada, Nasik City.

(*Flowering plants, their taxonomy and ecology.*)

1934 *Khanna, Lalit Prasad, M.Sc. (Punj.), F.L S , Rangoon University: Present address, Dept. of Botany, Ewing Christian College, Allahabad, U. P.

(*Plant Parasitic Nematodes and Liverworts.*)

1935 Kolhatkar, Govind Gopal, M.Sc. (Bom.), Assistant Professor of Botany, Fergusson College, Poona: Deccan Gymkhana Colony, Prof. Kolhatkar's Bungalow, Poona 4.

(*Pteridophytes and Angiosperms.*)

1939 Kumar, Krishna, M.Sc. (Benares), Assistant Professor of Plant Physiology, Institute of Agricultural Research, Benares Hindu University, Benares.

(*Plant Physiology, Genetics and Plant Breeding.*)

1936 *Kundu, Balai Chand, M.A. (Cal.), Ph.D. (Leeds), F L.S., Professor of Botany, Presidency College, Calcutta and Lecturer in Botany, Calcutta University, Calcutta.

(*Plant Anatomy, Charophytes, Mosses and Systematic Botany.*)

Date of election

1944 Lal, Akshaibar, M.Sc., Ph.D. (Lond.), D.I.C., Asst. Professor of Plant Pathology, Hindu University, Benares.

(*Plant Pathology, Plant Physiology and Crop Botany.*)

1944 Lal, Kashi Naresh, M.Sc., D.Sc., Lecturer, Institute of Agricultural Research, Benares Hindu University, Benares.

(*Plant Physiology and Plant Nutrition.*)

1939 LeGoc, Maurice Jacques, M.A. (Cantab.), B.Sc. (Lond.), Ph.D. (Rome), O.M.I., Vicar-General, Archbishop's House, Borella, Colombo, Ceylon.

1937 *Mahabale, Tryambak Shankar, B.A., M.Sc., Ph.D. (Bom.), Dept. of Biology, Gujarat College, Ahmedabad.

(*Living Cryptogams, Ferns and Morphology.*)

1927 *Maheshwari, Panchanan, D.Sc. (All.), F.N.I., Reader and Head of the Botany Department, Dacca University, Ramna P.O., Dacca.

(*Morphology and Cytology of Vascular Plants and Microtechnique.*)

1929 *Majumdar, Girija Prasanna, M.Sc. (Cal.), Ph.D. (Leeds), Professor of Botany, Presidency College, Calcutta and Lecturer in Botany, Calcutta University; 19, Ekdalia Place, Ballygunge, Calcutta.

(*Plant Anatomy.*)

1943 Malkani, Mrs. Sati A., M.Sc., Professor of Biology, D. G. National College, Hyderabad, Sind.

1945 Mehrotra, Anant Prasad, M.Sc. (Alld.), Research Scholar, Empress Victoria Reader, Dept. of Botany, University of Allahabad, Allahabad.

(*Plant Physiology.*)

1926 Mehta, Rai Bahadur Karam Chand, M.Sc. (Punj.), Ph.D., Sc.D. (Cantab), F.N.I., Professor of Botany, Agra College, Agra.

(*Mycology.*)

1939 Mehta, Khushi Ram, M.Sc. (Punj.), Assistant Professor of Botany, Benares Hindu University, Benares.

(*Anatomy, Palæobotany.*)

1935 Misra, Parasuram, M.Sc. (Cal), Ph.D. (Leeds), Head of the Department of Botany, Ravenshaw College, Chauliaganj P.O., Cuttack.

(*Plant Physiology and Cryptogams.*)

1942 Misra, Ramdeo, M.Sc. (Benares), Ph.D. (Leeds), Assistant Professor of Botany, Benares Hindu University, Benares.

(*Ecology, Physiology and Plant Geography.*)

1928 Mitra, Ajit Kumar, M.Sc. (Luck.), Ph.D. (Cantab), Economic Botanist to the Government of U.P., Sugarcane Research Farm, Nawabgunj, Cawnpur.

Date of election

1945 Mitra, Anil Kumar, B.Sc. (Hons.), M.Sc. (Alld.), Lecturer in Botany, University of Allahabad, and 22, K.P. Kakkar Road, Allahabad.

(*Algæ, Fungi, Plant Pathology.*)

1943 Mitra, Miss Eva, M.A., Ghosh Research Scholar in Botany, Calcutta University; 14, Chowringhee Terrace, Elgin Road P.O, Calcutta.

(*Systematic Botany.*)

1920 Mitra, Rai Bahadur Sochindra Nath, B.Sc., Dy. Conservator of Forests, Divisional Forest Officer, Sundarbans Division, P.O. Khulna, Bengal.

1925 Monica, Mother M., B A., L T., Professor of Botany, Loreto House, 7, Middleton Row, Calcutta.

(*Algœ and Palæobotany.*)

1942 Mooney, Herbert F., M.A. (Oxon.), I.F.S., Forest Adviser, Eastern States, Sambalpur (Orissa).

(*Systematic Botany and Ecology.*)

1943 Mukerjee, Susil Kumar, M.Sc. (Cal.), Ph.D. (Edin.), Curator of the Herbarium, Royal Botanic Garden, P.O. Botanic Garden, Sibpur, Howrah.

(*General Morphology, Taxonomy and Systematic Botany*)

1942 Mukherjee, Subodh Gopal, M.Sc., Lecturer and Acting Head of the Department of Botany, Jagannath Intermediate College, Dacca.

(*Physiology and Plant Anatomy.*)

1943 Mukherjee, Sunil Kumar, M.Sc. (Cal.), Assistant Cytologist, Mango Research Scheme, Dept. of Botany, Calcutta University, 35, Ballygunge Circular Road, P.O. Ballygunge, Calcutta.

(*Systematics and Cytology.*)

1937 Mulay, Babu Narhar, M.Sc., Ph.D. (Bom.), Assistant Professor of Biology, D. J. Sind College, Karachi.

(*Pteridophytes, Gymnosperms and Genetics.*)

1931 Mullan, D. P., B A., B.Sc. Hons., Vakil Terrace, 358, Lamington Road, Bombay.

1943 Murthy, Saragur Narasimha, B.Sc. (Hons.), M.Sc., 958, Lakshmipuram, Mysore (South India).

(*Morphology and Cytology.*)

1943 Nag, Prabhat Chandra, B.Ag. (Bom.), B.A., B.L., Agricultural Inspector, Howapara, Sylhet, Assam.

(*Physiology, Mycology, Systematic Botany.*)

Date of election

1939 Nandi, Hirendra Kumar, M.Sc. (Cal.), Ph.D. (Lond.), F.L.S., Economic Botanist to the Government of Assam, Shillong, Assam

(*Genetics, Cytology, Plant Breeding, Plant Physiology, Anatomy and Sytematic Botany.*)

1931 Nirula, R. L., B.Sc. Hons. (Punj.), Ph.D. (London), D.I.C., Head of the Department of Botany, College of Science, Nagpur.

(*Plant Pathology, Algæ and Floral Morphology.*)

1928 Oldroyd, Miss R. H., M.A. (Kansas State), Department of Botany, Isabella Thoburn College, Lucknow.

1945 Oza, Jayantilal Devshankar, M.Sc. (Bom.), Department of Biology, Gujarat College, Ahmedabad.

(*Mycology, Plant Pathology, Plant Anatomy.*)

1937 Pal, Benjamin Peary, M.Sc., Ph.D. (Cantab), F.L.S , Imperial Economic Botanist, Imperial Agricultural Research Institute, New Delhi.

(*Plant Genetics, Plant Breeding and Charophyta.*)

1945 Pal, Dr. N. L., D.Sc. (Alld.), Lecturer in Botany, University of Allahabad, Allahabad and Canning Road, Madhoapur, Allahabad.

(*Plant Physiology.*)

1922 Pande, Shiva Kant, M.Sc. (Punj.), D.Sc. (Luck.), Lecturer in Botany, The University, Lucknow.

(*Bryology, Cytology and General Morphology.*)

1944 Pantulu, Jayanti Venkanna, M.Sc., Demonstrator in Biology, Maharaja's College, Vizianagram.

(*Cytology, Genetics and Cytoecology.*)

1921 *Parija, Prankrishna, B.Sc. (Cal.), M.A. (Cantab), D.Sc., F.N.I., I.E.S., Vice-Chancellor, Utkal University, Cuttack.

1945 Puri, Gopal Singh B.Sc. (Hons.), M.Sc. (Punj.), Ph.D. (Luck.), Research Assistant, B. O. C. Palæobotanical Research, Botany Department, Lucknow University, Lucknow.

(*Palæobotany and Systematic Botany.*)

1936 Puri, Vishwambhar, D.Sc. (Agra), Professor and Head of the Dept. of Biology, Meerut College, Meerut.

(*Anatomy, Morphology and Systematic Botany.*)

1939 Qazilbash, Nawazish Ali, M.Sc. (Punj.), Professor of Botany, Islamia College, Peshawar.

(*Flora of N.W.F. Province, Botany and Chemistry of Ephedra and Artemisia*).

1931 Raghavachari, M.S., M.A., Professor of Botany, St. Berchman's College, Chenganacherry, Travancore.

(*Ecology and Cryptogams.*)

Date of election

1931 Raghavan, Tiruviladur Srinivas, M.A. (Mad.), Ph.D. (Lond.), F.L.S., Professor and Head of the Department of Botany, Annamalai University, Annamalainagar.

(*Cytology and Morphology of Angiosperms.*)

1943 Raghava Rao, K. V., M.Sc. (Mysore), Demonstrator in Botany, Hindu College, Guntur, Madras.

(*Morphology of Angiosperms and Plant Physiology.*)

1930 *Raizada, M. B., M.Sc. (Alld.), Forest Research Institute, New Forest, Dehra Dun.

1936 Randhawa, Mohindra Sing, M.Sc. (Punj.), F.N.I., I.C.S., Secretary, Imperial Council of Agricultural Research. New Delhi.

(*Algæ.*)

1920 Ranade, S. B., B.A., M.Sc. (Bom.), Lecturer in Botany, Ismail College, Andheri, Jogeshwari P.O., Bombay.

1922 *Ranjan, Shri, M.Sc. (Benares et Cantab), Dr. és Sc. (Toulouse), Professor of Botany, The University, Allahabad.

(*Plant Physiology.*)

1943 Rao, C. Surya Prakash, M.Sc., Lecturer in Botany, Erskine College of Natural Sciences, Andhra University, Guntur, Madras.

(*Algology and Taxonomy.*)

1935 *Rao, H. Sitarama, D.Sc. (Luck.), Economic Botanist, Essential Oil Advisory Committee, Forest Research Institute, New Forest P.O., Dehra Dun.

(*Plant Morphology and Palæobotany.*)

1922 *Rao, L. Narayana, M.Sc., Ph.D., Professor of Botany, Central College, Bangalore (S. I.).

1937 Rao, Sitarama, B.A. (Andhra), M.Sc. (Benares), Department of Biology, Ramnarain Ruia College, Matunga, Bombay.

(*Morphology, Anatomy and Cytology of Angiosperms.*)

1944 Rao, Y. Sundar, M.Sc., Lecturer in Botany, Mrs. A. V. N. College, Vizagapatam (S. India).

(*Cytology and Embryology.*)

1935 Rapinat, A. S. J., Professor of Botany, St. Joseph's College, Teppakulam P.O., Trichinopoly.

(*Systematic Botany, Bryology, Lichens and Histology.*)

1942 Raychaudhuri, S. P., M.Sc. (Cal.), Assoc. I.A.R.I., Agricultural Officer, I.C.C. Scheme, Dept. of Plant Pathology, College of Agriculture, Poona 5.

(*Mycology and Plant Pathology.*)

1944. Reddy, K. M. K., M.Sc., Lecturer in Botany, Mrs. A. V. N. College, Vizagapatam, South India.

(*Embryology and Cytology.*)

Date of election

1920 *Sabnis, Trimbak Sitarama, M.A., D.Sc. (Bom.), I. A. S., Economic Botanist (Fibres, Millets and Oilseeds) to the Government of U. P., Agricultural Gardens, Nawabganj, Cawnpore.

(*Systematic Botany, Physiology, Anatomy, Teratology, Variegation and Genetics.*)

1939 Sachar, Gurcharan Singh, M.Sc. (Punj.), Ph.D. (London), D.I.C., "Ajit Lodge", Murree, Punjab.

(*Mycology, Plant Pathology and Algæ.*)

1945 Sadasivan, T. S., M.Sc., Ph.D. (Lond.) Director, University Botany Laboratory, Triplicane, P.O. Madras.

(*Plant Pathology, Plant Viruses, Mycology and Plant Physiology.*)

1920 *Sahni, Birbal, M.A., Sc.D. (Cantab), D.Sc. (Lond.), F.R.S., Prof. of Botany, The University, Lucknow.

(*Palæobotany.*)

1939 Saksena, Ram Kumar, M.Sc. (Benares), Dr. és Sc. (Paris), Reader in Botany, The University, Allahabad.

(*Fungi.*)

1940 Sampath, Srinivasachari, M.A. (Mad.), Assistant Professor, Institute of Agricultural Research, Benares Hindu University, Benares.

(*Plant Karyology, Plant Breeding and Genetics.*)

1928 Sarbadhikari, Prabhat Chandra, Ph.D., D.Sc. (Lond.), D.I.C., F.L.S., Professor of Botany, and Head of the Department of Botany, University of Colombo, Ceylon.

(*Plant Cytology, Anatomy and Genetics.*)

1939 Sawhney, Rai Sahib Kalidas, M.Sc. (Punj.), Director, Agricultural Research, H.E.H. the Nizam's Government, Hyderabad, Deccan.

(*Agricultural, Economic and Applied Botany.*)

1932 Sayeed-ud-Din, M., B.Sc. (Bom.), M.A. (Edin.), F.R.M.S., F.A.Sc., F.L.S., Professor of Botany, Osmania University, Hyderabad, Deccan.

(*Systematic Botany and Ecology.*)

1937 Sen, B., B.Sc. (Cal.), Director, Vivekananda Laboratory, Almora, U. P., India.

1944 Sen, Nirad Kumar, M.Sc., Lecturer in Botany, Presidency College, Calcutta.

(*Plant Physiology.*)

1935 *Sen, Pabitra Kumar, M.Sc., Ph.D. (Lond.), D.I.C., Physiological Botanist, Fruit Research Station, Sabour.

1935 Sen, Srish Kumar, Retired Extra Asst. Commissioner, 3/5, Puran Paltan, Ramna P.O., Dacca.

(*Systematic and Economic Botany and Ecology.*)

Date of election

1943 Sen Gupta, Jatis Chandra, M.Sc. (Cal.), D.Sc (Heidelberg), Senior Professor of Botany, Presidency College, Calcutta and Lecturer, Calcutta University, 41, Lansdowne Terrace, Kalighat, P.O., Calcutta.

(*Plant Physiology, Ecology, Algæ.*)

1941 Senaratna, S. D. J. E., B.Sc. (Lond.), Assistant in Systematic Botany, Department of Agriculture, Ceylon; and Sri Palee Hindagala, Peradeniya, Ceylon.

(*Systematic Botany, Morphology, Anatomy, Ecology and Genetics.*)

1920 *Sethi, Mehr Chand, M.Sc. (Punj.), Professor of Botany, Forman Christian College, Lahore.

(*Systematic Botany and Algology.*)

1921 Shevade, S. V., B.Sc., Professor of Biology, M.T.B. College, Surat.

(*Taxonomy, Ecology, Plant Sociology and Cytology.*)

1940 Shukla, Vidya Bhaskar, M.Sc., Ph.D. (Luck.), Asst. Professor of Botany, College of Science, Nagpur.

(*Palæobotany.*)

1944 Singh, Balwant, M.Sc., Forest Research Institute, New Forest, Dehra Dun, U.P.

(*Vascular Anatomy, Algæ and Fungi.*)

1935 Singh, Bhola Nath, D.Sc. (Benares), Late Irwin Professor of Agriculture, University Professor of Plant Physiology and Head of the Institute of Agricultural Research, Benares Hindu University, Benares.

(*Plant Physiology, Agronomy, Biochemistry and Agricultural Botany.*)

1923 *Singh, Thakur Chandra Narayan, M.Sc., D.Sc., Agricultural Expert and Botanist, Horticultural Research Institute, Padhye-Gardens, Nagpur-Ajni, C.P.

(*Morphology.*)

1945 Sinha, Saligram, M.Sc., Ph.D. (Lucknow), Reader in Botany, Agra College, Agra.

(*Mycology and Plant Pathology.*)

1939 Sircar, S. M., M.Sc. (Cal.), Ph.D. (Lond.), D.I.C., Lecturer in Plant Physiology in Calcutta University, 35, Ballygunge Circular Road, Calcutta.

(*Plant Physiology.*)

1945 Sitholey, Rajendra Varma, M.Sc., Ph.D. (Lucknow), Research Assistant, B. O. C. Palæobotanical Research, Botany Department, Lucknow University, Lucknow.

(*Palæobotany.*)

Date of election

1945 Srivastava, Girja Dayal, M.Sc. (Luck.), F.N.A.Sc., Lecturer in Botany, University of Allahabad, Allahabad.

(*Morphology of Phanerogams, Systematic Botany, Ecology.*)

1921 Stewart, R. R., M.A., Ph.D. (Columbia), Principal and Professor of Biology, Gordon College, Rawalpindi.

(*Systematic Botany of Kashmir and N. W. Himalayas.*)

1945 Subramanian, V., M.Sc., Agri. Bot, Research Student, Department of Botany, University of Allahabad, Allahabad.

(*Plant Physiology.*)

1944 Swamy, B. G. L., B.Sc. (Hons.), C/o D. V. Gundappa Esq., Basavanagudi, Bangalore, South India.

1945 Tiwari, Devi Datt, M.Sc., Research Scholar in Botany, Department of Botany, University of Allahabad, Allahabad.

(*Mycology.*)

1920 *Tiwary, Nand Kumar, M.Sc. (Alld.), Assistant Professor of Botany, Benares Hindu University, Benares.

(*Morphology, Ecology and Bryophytes.*)

1945 Tiwari, S. D. N, M.Sc. (Nag). Asstt. Inspector of Civil Supplies, Govt. of India, "Salma Manzil" 4132, Napier Town, Jubbulpore, C. P.

(*Systematic Botany and Plant Breeding.*)

1945 Trivedi, Bhim Shanker, M.Sc., Research Assistant, Botany Department, The University, Lucknow.

(*Palæobotany.*)

1937 Vaheed-ud-Din, Syed, M.Sc., Ph.D. (Minnesota), Plant Pathologist, H. E. H. the Nizam's Government, Agricultural Department, Hyderabad, Deccan.

(*Plant Pathology and Mycology.*)

1926 Vakil, Bomanji Naoroji, M.Sc., Adenwalla Mansion, 11, Chaupathy, Seaface, Bombay.

(*Ecology, Physiology, Mycolgy, Pathology and Systematic Botany.*)

1931 Varardpande, K. V., M.Sc. (Benares), Assistant Professor of Botany, College of Science, Nagpur.

1945 Varma, Jagdish Chandra, M.Sc., Research Scholar in Botany, 12 Chatham Lines, Allahabad.

(*Mycology and Cytology.*)

Date of election

1936 Venkateswarlu, Jillella, M.Sc. (Benares), Lecturer in Botany, Erskine College of Natural Sciences, Andhra University, Guntur, Madras.

(*Morphology and Cytology of Angiosperms, Pharmacognosy and Medicinal Plants.*)

1943 Venkateswarlu, V., M.A., Lecturer in Botany, Hindu College, Masulipatam, M. S. M. Ry.

(*Cytology and Anatomy.*)

1945 Verma, Girja Shanker, M.Sc. (Lucknow), Lecturer in Botany, Botany Department, Lucknow University, Lucknow.

(*Mycology and Plant Pathology.*)

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